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NOTICE

The following courses of instruction will be given by the Liverpool School of Tropical Medicine during 1911 :—

Full Course begins 6 January. Short Course begins 1 June.
Diploma Examination, 3 April. Certificate Examination, 29 June.
Full Course begins 15 September.
Diploma Examination, 11 December.

The full Course of Instruction is open to all qualified medical men, and the Examination to all students who have taken out this full course.

Fee for the full Course of Instruction—Thirteen Guineas.

Fee for the Diploma Examination—Five Guineas.

Fee for the Short Course of Instruction—Four Guineas.

Fee for the use of a School microscope during one term—Ten shillings and sixpence.

For prospectus and further information, application should be made to the Dean of the Medical Faculty, University of Liverpool.

The following have obtained the Diploma in Tropical Medicine of the University of Liverpool :—

Diploma in Tropical Medicine

Date of Diploma	
1904	Augustine, Henry Joshua
1904	Bennett, Arthur King
1904	Bruce, William James
1904	Byrne, John Scott
1904	Clayton, Thomas Morrison
1904	Dalziel, John McEwen
1904	Dee, Peter
1904	Greenidge, Oliver Campbell
1904	Hehir, Patrick
1904	Khan, Saiduzzafor
1904	Laurie, Robert
1904	Maclurkin, Alfred Robert
1904	McConnell, Robert Ernest
1904	Nicholson, James Edward
1904	Philipson, Nicholas
1904	Sharman, Eric Harding
1904	Thomson, Frank Wyville
1904	Walker, George Francis Clegg
1905	Anderson, Catherine Elmslie
1905	Brown, Alexander
1905	Caldwell, Thomas Cathcart
1905	Critien, Attilio
1905	Hooton, Alfred
1905	Hudson, Charles Tilson
1905	Illington, Edmund Moritz

Date of Diploma	
1905	Macfarlane, Robert Maxwell
1905	Maddock, Edward Cecil Gordon
1905	Moore, James Jackson
1905	Nightingale, Samuel Shore
1905	Radcliffe, Percy Alexander Hurst
1905	Young, John Cameron
1906	Adie, Joseph Rosamond
1906	Arnold, Frank Arthur
1906	Bate, John Brabant
1906	Bennetts, Harold Graves
1906	Carter, Robert Markham
1906	Chisholm, James Alexander
1906	Clements, Robert William
1906	Dundas, James
1906	Faichnie, Norman
1906	Jeffreys, Herbert Castelman
1906	Mackenzie, Donald Francis
1906	Pailthorpe, Mary Elizabeth
1906	Palmer, Harold Thornbury
1906	Pearse, Albert
1906	Sampey, Alexander William
1906	Smithson, Arthur Ernest
1906	Taylor, Joseph van Someron
1906	Taylor, William Irwin
1906	Tynan, Edward Joseph

Date of Diploma	
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1906	Willcocks, Roger Durant
1906	Williamson, George Alexander
1907	Allan, Alexander Smith
1907	Allwood, James Aldred
1907	Bond, Ashton
1907	Branch, Stanley
1907	Collinson, Walter Julius
1907	Davey, John Bernard
1907	Donaldson, Anson Scott
1907	Fell, Matthew Henry Gregson
1907	Gann, Thomas William Francis
1907	Graham, James Drummond
1907	Hiscock, Robert Carroll
1907	Keane, Joseph Gerald
1907	Kennan, Richard Henry
1907	Kenrick, William Hamilton
1907	Le Fanu, George Ernest Hugh
1907	Mackey, Charles
1907	Maddox, Ralph Henry
1907	McCarthy, John McDonald
1907	Raikes, Cuthbert Taunton
1907	Ryan, Joseph Charles
1907	Vallance, Hugh
1908	Caverhill, Austin Mack
1908	Crawford, Gilbert Stewart
1908	Dalal, Kaikhusroo Rustomji
1908	Dansey-Browning, George
1908	Davidson, James
1908	Dickson, John Rhodes
1908	Dowdall, Arthur Melville
1908	Glover, Henry Joseph
1908	Greaves, Francis Wood
1908	Goodbody, Cecil Maurice
1908	Harrison, James Herbert Hugh
1908	Joshi, Lemuel Lucas
1908	Le Fanu, Cecil Vivian
1908	Luethgen, Carl Wilhelm Ludwig
1908	Mama, Jamshed Byramji
1908	McCay, Frederick William
1908	McLellan, Samuel Wilson
1908	Pearce, Charles Ross
1908	Schoorel, Alexander Frederik
1908	Smith, John Macgregor
1908	Stewart, George Edward
1908	Tate, Gerald William
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1909	Abercrombie, Rudolph George
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1909	Henry, Sydney Alexander
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1909	Samuel, Mysore Gnanandaraju
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1910	Houston, William Mitchell
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EDITORIAL NOTICE

By order of the Committee of the Incorporated Liverpool School of Tropical Medicine, the series of the Reports of the School, which had been issued since 1899, were followed, from January 1, 1907, by the Annals of Tropical Medicine and Parasitology, of which this is the third number of the fourth volume.

Altogether twenty-one Memoirs, besides other works, were published by the School since 1899, and of these ten, containing 519 quarto or octavo pages and 95 plates and figures, were published during the two years 1904 and 1905.

The Annals are issued by the Committee of the School, and will contain all such matter as was formerly printed in the Reports—that is to say, accounts of the various expeditions of the School and of the scientific work done in its laboratories at the University of Liverpool and at Runcorn. In addition, however, to School work, original articles from outside on any subject connected with Tropical Medicine or Hygiene may be published if found suitable (see notice on back of cover); so that, in all probability, not less than four numbers of the Annals will be issued annually. Each number will be brought out when material sufficient for it has been accumulated.

SOME ENUMERATIVE STUDIES ON MALARIAL FEVER

BY

MAJOR RONALD ROSS, F.R.S.,

AND

DAVID THOMSON, M.B., CH.B., D.P.H.

(*Received for publication* 15 *November*, 1910)*

1. *Preliminary.* For many years past little information which is both new and exact has been added to our knowledge of the pathology of malaria. This has probably been due to the exhaustion of the older methods of research which, being purely qualitative, have failed to indicate the precise correlations between the numbers of the parasites present in a patient and the various pathological and therapeutical reactions. For example, out of fifty-one and thirty-eight successful inoculations of men by means of infected blood and infected Anophelines respectively, in not a single one has any exact estimate been given of the number of parasites inoculated or recovered after the lapse of the incubation period; and, though many researches on quinine have been made, we know of none in which its direct effect upon the numbers of the parasites in the patient has been correctly measured. Moreover, the older methods often failed to reveal the parasites at all, unless they were present in large numbers. Hence our first care was to elaborate more exact methods both for detection and for enumeration. This was done early in the year (by R.R. and D.T.), though we have by no means reached finality yet. The new methods were next employed for all the cases in the Tropical Ward, daily counts of the parasites and often of the leucocytes, together with other estimations, being made (by D.T.). At the same time parallel chemical studies were carried out and therapeutical ones, and the measurements were carefully charted and compared. Even at this preliminary stage the results (which are given at the end of this paper) include more accurate verifications of some old conjectures, and demonstrations of some new theorems.

* (Read before the Royal Society, Dec. 8th, and reprinted from Proc. Roy. Soc., Series B., Vol. LXXXIII, p. 159, with Addenda.)

2. *The detective method used.* In the ordinary methods the blood is spread out in a thin film, liquid or dry, one cubic millimetre covering say four square centimetres of area. If the field of the oil-immersion lens has a diameter of 0·165 mm. and can be thoroughly searched by the eye in three seconds, about twelve hours' work would be required to examine the whole of 1 c.mm. of blood in this way; and if the specimen contains only one parasite it may not, by bad luck, be found until nearly the whole of the area has been searched. We have therefore employed the 'thick film process' described by one of us (R.R.) in 1903. In this 1 c.mm. of blood is spread out over only about one-quarter of a square centimetre or less, and dried; the haemoglobin is then gently washed out with water; and the residue, consisting of parasites, leucocytes, platelets, and the stromata of the red corpuscles, is carefully stained by any convenient method, with or without fixation. By this simple means the whole of a cubic millimetre of blood can be searched in less than an hour; but the identification of the Plasmodia requires considerable practice.

3. *The enumerative method used.* The younger *Plasmodia* are too small to be counted by the use of an ordinary haemocytometer; hence their number has usually been estimated by comparison with the number of red or white cells, the latter figure being determined by the haemocytometer. This gives a large compound error; for if e is the percentage error made in counting the red or white cells in one specimen, and e^1 is the percentage error made in computing the proportion of parasites to each corpuscle in that specimen, then, by a simple calculation, the total percentage error will be $e + e^1 + ee^1/100$. (For example, if $e = +5\%$ and $e^1 = +10\%$, the total percentage error will be $+15{\cdot}5\%$.)

Our method consists simply in making a measured quantity of blood into a thick film preparation and then counting *all* the parasites in it. The blood is measured by determining the diameter of a fine capillary tube under the microscope, and then calculating and marking off the length of the tube required to hold a given quantity (as suggested by Dr. Wakelin Barratt). Thus a uniform tube of 0·180 mm. diameter and 4 cm. length will contain 1·018 c.mm.—a convenient size. The blood is sucked into the tube

and then quickly discharged upon the glass-slide in one or several measured droplets, first samples being rejected. The dehaemoglobinized thick film is fixed before staining in order to avoid loss, and the whole area is scrupulously searched with the aid of a sliding stage.

Mr. M. Greenwood, Junr., informs me that if n is the number of parasites counted in the unit (say 1 c.mm.) of blood, and m is the number of such units in the patient's whole body, then the total number of parasites in him, assuming uniform distribution, will be $mn \pm 0{\cdot}67449m\sqrt{n}$. Thus the probable percentage error is $67{\cdot}449/\sqrt{n}$, the factor m cancelling out from the ratio. This is important, because it shows that the error depends, not on the magnitude of the measured sample taken, but upon the number of parasites actually counted in it. Hence, in order to obtain results within an assigned error, we take a large sample when the parasites are scarce and a small one when they are numerous; the number of parasites which we must find and count being given by the formula $n = 4550/e^2$, where e is the permissible error.

The greatest error occurs when the parasites are so scarce that it is difficult to find enough of them to count up to the required standard; or when they are so numerous that it is difficult to measure a volume of blood small enough to contain an easily countable number of them. In these preliminary researches we have seldom examined at one sitting more than 1 c.mm. of blood, or less than $\frac{1}{4}$ c.mm.

When the parasites were very numerous we have sometimes fallen back on estimating their number by comparison with leucocytes, but are now elaborating improvements and special instruments for such, and other, details. The method is also accurate and rapid for other blood parasites, and for leucocytes. Other sources of error are (1) inaccurate measurements of the quantity of blood used, and (2) difficulty of seeing or distinguishing badly stained parasites.

One kilogram of blood of average density (1057·5) contains 945,626 cubic millimetres, and the blood in a man's body is estimated to weigh about 4·9 per cent. of the total body weight, from which data it is easy to compute the total number of parasites in a patient. A man of 64·74 kilograms, or about ten stone, will contain about 3,000,000 c.mm. of blood.

4. *The cases studied* by us numbered thirty-three. All had been infected in West Africa or America, so that none was of less than some weeks' duration when admitted into hospital in Liverpool. All were males of from eighteen to sixty years of age. They were mostly shipmen or traders, and two were negroes. The parasites of all were counted almost every day, and sometimes several times a day; and the temperatures were taken every four hours, or more often. One case showed *Plasmodium malariae*, together with crescents (sexual forms of *P. falciparum*). Eight showed *P. vivax* only, and twenty-four *P. falciparum* only, two of the latter containing only sexual forms. Quinine was often withheld for some days while other methods of treatment were used. The cases were studied for 600 days altogether. There were no deaths.

All the cases are tabulated at the end of the paper for verification of details, and charts of cases 17 and 20 are given. At this preliminary stage of the enquiry we have undertaken to study only the gross daily correlations between the numbers of parasites and other phenomena, leaving more minute hourly and four-hourly analysis to future work. Hence if a number of counts or other observations have been made on one day we have recorded only the averages in the tables. For temperatures, however, it has been thought best to record maxima instead of averages, and, as the tables should be as economical as possible, we have used the *haematothermic scale* suggested by one of us (R.R.), in which the ten degrees between 95° and 105° F., or between 35° and 45° C., are divided into 100 parts. Except Case 6, specially studied by Dr. Fantham, we have not distinguished in the tables the various forms, sets or stages of *P. vivax*, this being reserved for future study. Similarly, the treatment, leucocytes, haemoglobin and urobilin are not differentiated too minutely. Case 25 was counted by Dr. Korke.

5. *The correlation between the parasites and the fever.* It is well known that fever is caused only by the asexual forms of the parasites; but though these are generally thought to be more abundant during pyrexial periods, no extensive numerical studies on the point appear to have been made, with the result that some sceptics still profess doubts on the subject. Our cases, except four without fever (Cases 1, 15, 16, 27), would seem to

indicate a very strong, almost convincing, correlation. Twenty-one of them suffered each from one pyrexial period, lasting from one to seven days and preceded or followed by apyrexia; and in all except Case 32, the asexual parasites were very much more numerous during the pyrexial period. Nine of the cases (7, 17, 18, 19, 20, 23, 24, 26, 32) suffered from one or two relapses each (thirteen relapses altogether); and every relapse, as well as every original attack, was associated with a comparatively large number of parasites (forty-three pyrexial periods altogether). Similarly, there were forty-six apyrexial periods (including the four cases without fever), all associated with comparatively small numbers of asexual parasites; and conversely not a single marked rise in the number of asexual parasites took place without corresponding fever.

For further details we compare the numbers of parasites per c.mm. counted on days of fever with those counted on days without fever. A febrile day is taken as any one on which the patients' temperature exceeds 98·6° F. (=36° Haematothermic Scale), or any one which comes between two tertian paroxysms (Cases 9, 14, 24). The non-febrile days on which no asexual forms could be found in 1 c.mm. of blood are omitted. The results are:—

	Cases	Days	Total parasites	Average per day	Ratio
P. vivax—					
Fever	8	30	136,062	4,535	36·3
No fever	8	68	8,487	125	—
P. falciparum—					
Fever	21	97	1,319,880	13,607	29·6
No fever	19	83	38,277	461	—

If we had not omitted the non-febrile days on which no parasites were found, the non-febrile daily averages would have been much smaller than the figures given above, and the ratio of the febrile averages to them much larger; so that the febrile excess is very marked. An examination of the details will further convince the reader of the great fall in temperature which accompanies the fall

in the number of parasites. We should note that the comparatively small numbers of *P. vivax* found are due to the fact that the specimens generally contained a large proportion of mature parasites, not as yet disintegrated into spores—these, of course, being absent from peripheral blood containing *P. falciparum*, in which only spores or young forms generally occur. There is no reason for considering quinine in this connection, because it probably affects the fever only through its action on the parasites.

6. *The degree of fever associated with various numbers of parasites.* We know of no extensive and exact studies on this point, and ours are not yet complete. On forty-seven occasions the number of parasites was carefully compared with the maximum degree of temperature reached during the febrile paroxysm caused by them, and the following results were obtained:—

P. vivax (12 occasions)—						
No. of parasites	1,500	1,400	580	440	430
Maximum temp. °H.F. * ...	84	101	97	102	76
No. of parasites	260	256	232	150	133
Maximum temp. H.F.	76	54	46	44	34
No. of parasites	100	83			
Maximum temp. H.F.	34	20 (rigor)			
* Co-efficient of correlation r	= 0·68561	± 0·10318			
P. falciparum (35 occasions)—						
No. of parasites	303,000	58,000	55,000	54,000	50,000
Maximum temp. °H.F.	108	82	88	100	98
No. of parasites	45,000	36,000	34,000	31,000	26,000
Maximum temp. H.F.	76	60	86	76	94
No. of parasites	26,000	25,000	16,000	15,000	15,000
Maximum temp. H.F.	101	80	52	54	54
No. of parasites	15,000	9,700	9,000	7,500	7,400
Maximum temp. H.F.	70	56	70	90	40
No. of parasites	6,700	6,500	6,000	5,800	5,200
Maximum temp. H.F.	86	83	52	70	76
No. of parasites	4,000	4,000	3,600	3,000	2,600
Maximum temp. H.F.	70	40	80	38	64
No. of parasites	2,500	2,000	1,860	1,000	200
Maximum temp. H.F.	50	46	38	15	50
Co-efficient of correlation r	= 0·60331	± 0·0·251			

* H.F. = Haematothermic scale Fahrenheit.

These figures, together with the numerous ones given in the table of cases, show a very marked correlation, but there are, of course, many minor deviations. In numbers of the specimens there were certainly two sets of parasites of different ages, which were often counted together, though only one set could have been concerned with the fever. It is probable also, though by no means certain, that the resistance to the toxin of the *Plasmodia* varies, not only in different persons, but in the same person at different stages in the course of his infection and under different physiological conditions. Our new methods will, we hope, enable us shortly to undertake very detailed studies on these points.

7. *The pyrogenic limit.* It is quite evident that if the parasites fall below a certain limit they are no longer numerous enough to cause fever. Thus with *P. vivax*, an average of 125 parasites of all ages and forms counted during sixty-eight days in eight cases, and with *P. falciparum* an average of 460 asexual forms counted during eighty-three day in nineteen cases, failed to produce fever. The actual limit above which they become pyrogenic probably varies in different cases. With *P. vivax* as many as 1,500, 852 and 540 were found in Cases 2, 6, and 7 without fever, and as few as 150 and 50 in Cases 5 and 9 with very slight fever (98·6° F.), so that about 200 to 500 may perhaps be taken as the usual limit. With *P. falciparum*, as many as 1,620 (asexual) were found as an average of four non-febrile days in Case 26, and 1,196 as an average of six non-febrile days in Case 25; while in Case 18, from 16 to 1,860 were present for twenty-two days without causing any marked fever, so that 600 to 1,500 may perhaps be adopted as the usual limit. With *P. malariae* (Case 1), an average of 79 were present for thirteen days without fever, but about 140 twice caused slight rigor.

8. *The asexual forms between the relapses.* It is generally thought and taught that these forms tend to 'disappear' between the relapses—though of course exceptions, especially in children, are noted—and to reappear during them; and several hypotheses, such as that of parthenogenesis (F. Schaudinn) have been advanced explain the supposed phenomenon and have led to what appears to be much waste of work. We find that the asexual forms do not

necessarily disappear at all between the relapses, but are generally still to be found in small numbers per c.mm. on most of the days. The apyretic periods preceding the thirteen relapses in our cases (7, 17, 18, 19, 20, 23, 24, 26, 32) lasted 114 days altogether (nearly nine days each on the average). On eighteen of these days no observations were made; but on the remaining ninety-six days the parasites were found in small numbers in 59, or 61 per cent., and were not found only in 37. Moreover, the general trend of the curves suggests that they were not found on these days only because their numbers were a little too few for detection. The parasite curve, at its height during a pyrexial period, generally falls very rapidly at first and more slowly later, and tends to reach its lowest about half way between two apyrexial periods. At this point it may or may not remain above the detectable limit (by thick film methods). After this it was observed (by D.T.), especially in Cases 7, 17, 23 and 24, to begin mounting slowly at first, until when it reached the pyrogenic limit, another pyrexial period commenced. All this is scarcely compatible with the speculation that the apyrexial periods are due to the abrupt death of most of the asexual *Plasmodia*, or to their conversion into 'resting stages,' etc. Nor do such speculations appear to be at all necessary. It is easy to see that the survival of comparatively small numbers of the asexual forms will suffice to keep the infection alive, not only for the short periods observed by us, but for 'relapses of long interval,' and for months or years. Obviously, if only a few parasites per c.mm. are present, they may easily be overlooked in the small amount of blood (say 0·1 c.mm.) usually examined by thin film, and may then be reported as being absent: yet many millions may still exist in a patient who contains say 3,000,000 c.mm. of blood. The speculation regarding parthenogenesis in malaria and the case of Schaudinn, supposed to support it, have been criticised elsewhere by one of us (R.R.).* In two of our relapsing cases (19, 26) no sexual forms at all were found during thirty-five different thick film examinations. Even if they were present in small numbers (crescents do not collect in the spleen), yet the numbers of asexual parasites found in or just before the relapses (24 and 400 per c.mm.) cannot be explained by parthenogenesis unless we suppose that each

* Prevention of Malaria (Murray, 1910).

sexual form produced by 'gametoschizogony' 840 or 14,000 spores. Until better evidence for such views is adduced, our results justify the doctrine that the malarial infection is kept continuously alive simply by the persistence of the asexual form in varying numbers; and that fever occurs only when the forms are numerous enough to produce it.

9. *The effect of quinine on the asexual forms.* The destructive effect of the drug is of course everywhere recognised, though very little completely satisfactory statistical evidence can be cited in proof. Many laborious researches have been made regarding the comparative utility of the various salts, but these have been confined almost entirely to estimating the rate and percentage of absorption, judged from urinary elimination. The subject is of the greatest sanitary and medical importance; but, so far as we can see, it can be usefully studied only by the more detailed enumerative analysis which we propose soon to undertake. Our daily analysis gives little more light; but the following figures may be mentioned. Cases 15, 16 showed no asexual forms; Cases 11 and 13 were so severe that quinine was given at once; Cases 1, 5, 27, 31 and 32 were so mild that quinine was withheld during the period of observation; and in the remaining cases the drug was withheld only for some days (except an occasional necessary dose) and was then given continuously while the parasites were being counted. We have therefore added together all the daily counts during the periods of no-quinine and quinine respectively. With eight cases of *P. vivax* there were forty-five no-quinine days showing 104,032 total parasites, or an average of 2,312 a day; and there were 74 quinine days with 31,769 total parasites, or an average of only 429 a day (one-fifth). With *P. falciparum* (19 cases), 147 no-quinine days showed an average of 31,136 asexual forms, and 148 quinine days an average of 1,535 (one-half). We should have expected that the proportion of quinine-day parasites would have been smaller; but the no-quinine counts are diminished by the inclusion of the five mild cases and also by doses of 10 to 20 grains which were given on ten isolated occasions. On quinine days the doses are generally 20 to 30 grains a day, and Cases 11, 13, 14, 15 and 16 and parts of other cases are omitted because they were unsuitable. Great falls in the number of parasites occurred, even

without quinine, in Cases 5, 17 and 26; while, on the other hand, small numbers of asexual forms remained in some cases for five or more days in spite of considerable daily dosage. In Case 23 a severe relapse began six days after the stoppage of quinine tannate, which had been given for nine previous days in ten to thirty grain doses daily. No other relapses occurred after continuous quinine treatment; but twelve occurred without it.

10. *Observations on the sexual forms.* It is, of course, generally held—and probably quite rightly—that these are developed (by a cytological process not yet clearly seen) from the asexual forms. But at the same time no one has been able to note any correspondence between their numbers—many of the former may be present when the latter are very scarce, and vice versa. This rule, which is fully confirmed by our figures, has always been difficult to reconcile with the accepted theory of origin. On comparing our curves, however, the remarkable fact was observed (by D. T.) that there often seems to be indeed a correspondence, but that it is delayed for from eight to ten days—that is, that the sexual curve tends to rise that number of days after a rise in the asexual curve. Out of our twenty-five infections with *P. falciparum* eighteen showed crescents, and of these cases, 14, 22, 17, 18, 20, 23, 24 and 30 suggest this phenomenon, while in 17 and 20 the rises are sufficiently isolated to show it most distinctly. In Case 17, asexual rises existed on the 1st-3rd days, the 11th-12th and the 21st-22nd days, separated by apyrexial periods during which few asexual and no sexual forms were found; but on the 30th day (eight days after the last relapse) crescents appeared for the first time. In Case 20 an asexual relapse commenced on the 17th day and reached its height on the 20th day. A very few crescents had been found all the time; but a marked rise in their numbers commenced on the 26th day and reached its height on the 28th day. The most ready explanation is that the *crescents require eight to ten days for development*, during which they remain undetected, probably in the internal organs of the host. This is confirmed by the fact that numerous small ones are seen at the commencement of a rise; and also by the observation (frequently made) that in fresh infections the crescents seldom appear until a

week or more after the first attack of fever. Strong confirmation is also given by Case 6, in which Dr. Fantham noted a sudden appearance of sexual forms (*P. vivax*) on the 11th day, seven to ten days after the original asexual rise and after the patient had been taking thirty grains of quinine daily for a week—suggesting that the same phenomenon holds for *P. vivax* also.

Other observations are as follows:—(1) By no means every asexual rise is followed by a sexual one. In seven cases (13, 19, 25, 26, 28, 31, 33) no crescents at all were seen. In Case 13, a negro, 173,400 asexual forms per c.mm. failed to produce any; and none was observed in another negro (Case 28). (2) The number of crescents never exceeded five per cent. of asexual forms found in the generation which (hypothetically) produced them, and were generally in much smaller proportion. (3) We could find no relation to youth, vigour, amount of haemoglobin, duration of infection (from three to thirty weeks), or season of first infection. (4) The daily counts of crescents generally show marked daily variations, but we think that after considerable doses of quinine have been given for some days these daily variations tend to be smoothed out (Cases 16, 18, 22, 24). (5) In Cases 15, 14 and 16, particularly, a distinct tertian tendency was observed in the daily variations of crescents. This appears not to have been previously noticed, but is just what would be expected in the case of a tertian parasite—though, of course, the curve would be confused in a double tertian. (6) Sometimes the crescent curve suddenly rises with great rapidity, reaches its maximum, and then begins to fall on the next day with equal rapidity, though, later, the fall often tends to be much more slow (Cases 14, 15, 22, 23), especially when the numbers are very small. Apparently, the greater the maximum the quicker fall (Cases 14, 15); and the fall is sometimes jagged with a tertian tendency (ibid.). (7) Sometimes the maximum is irregularly maintained for a number of days (Cases 16, 18, 24), especially when quinine has not been previously given. (8) The crescents remained detectable for as many as 31, 32, 32, 35 and 44 days in Cases 20, 22, 23, 24 and 18 respectively; and finally disappeared (in 1 c.mm. of blood) in Cases 1, 11, 12, 16, 21 and 23.

From these data we think (1) that a varying percentage of asexual forms (sometimes very few or none) are constantly

generating crescents, which, after about eight to ten days, appear in the peripheral blood. Hence, when the asexual forms remain sufficiently numerous for a period, as when no quinine is given, the stock of crescents is being constantly replenished. Thus the mortality among the older crescents is constantly being compensated for by new arrivals, and the total numbers appear to remain constant—an appearance which has given rise to the generally accepted hypothesis (perhaps erroneous) that the crescents survive for weeks. On the other hand the sudden rise and fall of their numbers seen in Cases 14, 15, 22 and 23, suggest that they really survive only for a short time after their appearance in the peripheral blood. In Cases 16 and 24, however, a few crescents were still found after quinine had been given continuously for sixteen to eighteen days, suggesting that some of them may be able to survive for longer periods. In Cases 20, 22 and 23, they appeared in increasing numbers for four to six days after continuous 30-grain doses of quinine had been commenced, suggesting that the drug has no effect upon them when they have once been generated. But quinine appears to affect the numbers of crescents indirectly by cutting off the source of supply. Though many researches upon the effect of quinine on crescents (a subject of the greatest sanitary importance) have been attempted, so far as we know the possibility of a constantly continued supply has not previously been taken into consideration. We are continuing researches on the point.

11. *The leucocytes* were counted frequently, but not always daily; at first by the haemocytometer, but in the later twenty cases by the thick film method. The latter method has the advantages of putting the leucocytes closer together and of enabling us to estimate them at the same time with the parasites; but, like the haemocytometer, it is not very appropriate for differential counts because of occasional uncertainty in distinguishing the various kinds. Accordingly these were made (in six cases) by thin films, 500 cells being examined on each occasion.

Our results are, as shown in all the cases, that during the pyrexial periods the total leucocytes are comparatively few, being frequently as low as 2 to 3,000 per c.mm. (Cases 17, 18, 23, 24. etc.). But as

the asexual forms and the fever diminish the total leucocyte curve rises, and about seven days later exceeds the normal and may reach 20,000 and even, rarely, 30,000 per c.mm. (Case 7)—though this height is not maintained. The very high counts seem to be associated with much quinine. We observed generally that the so-called polymorphonuclear leucocytes, though rather few during the pyrexial periods, do not vary much from day to day, but about seven days after the fever has abated their numbers are markedly increased and vary much. The so-called mononuclear percentage was, however, always in excess throughout the cases, even long after disappearance of all forms of the parasites—for six weeks afterwards in Case 1, though quinine had been given daily during that period (out of hospital). We think, therefore, that a high mononuclear percentage is likely to be always of value in diagnosis in the absence of parasites. The normal ratio of all mononuclears to polymorphonuclears is about 35 per cent., but in our cases it was often 60 per cent., and in Case 30 reached 80 per cent. It tends to fall slightly with great improvement in health. Thus the total leucocyte rise appears to be due chiefly to mononuclears. During the individual daily paroxysms, as shown by Stephens and Christophers,* the total mononuclears are rather low during the height of the fever, but show a marked increase during the following remission, this increase affecting chiefly the large mononuclears. This process is repeated with each paroxysm, and if a paroxysm fails the mononuclears remain increased (Case 9 especially). But we could not always find these variations in the absence of fever and many parasites. This mononuclear reaction, which we have also seen in our case of trypanosomiasis, appears to be exactly comparable to the polymorphonuclear reaction described by F. W. Andrews ('Lancet,' June, July, 1910) as generally occurring in bacterial diseases.

12. *Various therapeutic agents.* Methylene blue was tried in three cases in 12 grain daily doses; the parasites diminished, but we cannot draw any conclusions from the figures. In Case 24, altogether 36 grains of soamin were injected intramuscularly in

* The increase in the number of large mononuclear leucocytes as a diagnostic sign of malaria. Royal Society's Malaria Committee Reports, Fifth Series, 1901. Harrison & Sons, London.

5 to 10 grain doses on five occasions during twelve days, and yet a smart relapse occurred during and after the last dose. In the same case just before the same relapse X-rays had been applied over the spleen and abdomen for twenty minutes. The asexual parasite rise had commenced before the application. In Case 24, faradic and galvanic currents were applied over the spleen, each for ten minutes, but crescent counts made immediately before and two hours after the application remained unaltered

13. *Haemoglobin.* This was estimated in most of the later cases. The percentage always fell during fever and began to rise rapidly shortly afterwards, proving the destructive effect of the parasites and the rapid recuperation of the patients. The greatest fall was 25 per cent. after four days' fever (Case 20); but more detailed work is required to trace correspondence with the number of parasites. The lowest percentage found was 53 per cent. All the patients were anaemic at first, but the haemoglobin rose rapidly with improvement of health, and very rapidly in the more vigorous subjects. We decided not to attempt counts of red corpuscles, as these have been made so frequently already. The elimination of the haemoglobin and the findings in a case of blackwater fever will be dealt with in the two following papers by Dr. Simpson and in one by ourselves.

14. *Summary.* (1) There would seem to be a very decided correlation between the number of asexual *Plasmodia* found in the peripheral blood and the fever.

(2) As a rule, no fever exists unless the asexual forms exceed some hundreds per c.mm.

(3) The asexual forms do not always disappear between relapses (as often thought) but tend to persist in small numbers per c.mm., and often increase again for some days before the actual febrile relapse occurs.

(4) These observations give a coherent theory of the malarial invasion, according to which the infection is kept alive indefinitely by the ordinary sporulation of the asexual forms, and not by parthenogenesis or by resistant forms; and fever recurs only when the parasites are numerous enough to produce it.

(5) We estimate from our cases that considerable continued doses of quinine reduced the asexual forms by 50 to 80 per cent.

(6) There are strong reasons for supposing that the sexual forms require eight to ten days for development; that the often noticed long persistence of crescents is not due to their long life (as generally thought) but to constant replenishments of the stock by fresh broods; that they sometimes show a distinct tertian periodicity; and that quinine does not affect them when once generated; but ultimately reduces their numbers by destroying the generating cells. The sexual forms were never seen to produce fever.

(7) The leucocytes are below normal during febrile periods and above normal afterwards. The percentage of mononuclears rises after paroxysms and is always in excess of the normal.

(8) Methylene blue, soamin, X-rays, and faradic and galvanic currents had no results in a few experiments.

(9) The haemoglobin falls markedly with fever, but rises rapidly with convalesence.

(10) The faecal urobilin shows marked correlation with the occurrence of fever, and is specially studied in the accompanying paper by Dr. G. C. E. Simpson.

Many other deductions may be based on our observations, but will be better considered after more detailed counts have been made.

TABLE GIVING THE DAILY DETAILS OF 33 CASES OF MALARIA STUDIED BY ENUMERATIVE METHODS

NOTE.—The headline of each case gives initials, calling, age, where infected (presumably), how long previously infected (presumably), month of admission, and body weight on admission. The maximum temperatures are given in the Haematothermic Fahrenheit scale (H.F.), which equals the excess over 95°F., multiplied by 10. The leucocytes are recorded in hundreds per c.mm.; the haemoglobin either in the Talqvist or the Sahli scale; the urobilin (either urinary or faecal) in milligrams; the drugs in grains; and the parasites (either all forms together or asexual and sexual forms separately) in numbers per c.mm.

A. *P. malariae* and *P. falciparum* (crescents only).

Case 1.—W. M., ship butcher, 42; West Africa; 70 days; February, 145 lbs.

Days	1	2	3	4	5	6	7
Total parasites	—	254	76	—	148	121	151
Crescents	200	164	170	—	180	141	156
Maximum temp.	26	20 (rigor)	20	22	30	34 (rigor)	30
Methylene blue, grs.	—	—	—	—	4	12	12
Polychr. methylene blue, c.c.	—	—	—	—	—	—	—
Ur. urob., mgr.	20	?	18	0	0	—	—

Days	8	9	10	11	12	13	14
Total parasites	127	140	57	17	17	23	8
Crescents	220	117	86	70	51	38	16
Maximum temp.	40	30	30	30	16	22	34
Methylene blue, grs.	12	12	12	12	12	12	0
Polychr. methylene blue, c.c.	—	—	—	—	—	—	—
Ur. urob., mgr.	—	—	—	—	—	—	—

Days	15	16	17	18	19	20	21
Total parasites	16	6	0	—	0	0	0
Crescents	10	10	0	—	2	2	2
Maximum temp.	34	30	34	24	30	30	22
Methylene blue, grs.	—	—	—	—	4	12	12
Polychr. methylene blue, c.c.	15	45	30	0	—	—	—
Ur. urob., mgr.	—	—	—	—	—	—	—

B. *P. vivax.*

Case 2.—J. B., seaman, 26; Colon; 120 days; January, 147 lbs.

Days	1	2	3	4	5	6	7
Total parasites	8,766	6,233	2,630	1,500	1,260	—	0
Maximum temp.	48	38	58	20	24	24	18
Q. hydrochloride, grs.	0	15	30	30	30	30	30

Case 3.—J. H., seaman, 50; W. Africa; 35 days; January, ?

Days	1	2	3	4	5	6 to 11
Total parasites	11,019	1,444	642	88	40	0	0
Maximum temp.	84	42	44	34	32	22	normal
Q. sulph., grs.	—	—	—	—	20	20	20
Ur. urob., mgs.	2	0	0	0	0	—	—

Case 4.—J. Hough, ship steward, 22; Vera Cruz; 112 days; March, 105 lbs.

Days	1	2	3	4	5	6	7
Total parasites	370	308	505	280	46	8	0
Maximum temp.	46	44	76	58	30	26	28
Q. hydrochloride, grs.	—	—	—	30	30	30	30
Total leucocytes	—	55	—	86	—	—	—
Hb. Tal., percentage	90	85	85	80	85	85	85
Ur. urob., mgs.	54	0	18	82	140	17	19
Days	8	9	11	—	—	—	—
Total parasites	0	0	0	—	—	—	—
Maximum temp.	20	24	14	—	—	—	—
Q. hydrochloride, grs.	30	30	30	—	—	—	—
Total leucocytes	84	—	160	—	—	—	—
Hb. Tal., percentage	85	85	90	—	—	—	—
Ur. urob., mgs.	0	0	—	—	—	—	—

Case 5.—R. R., planter, 25; Fernando Po; 75 days; March, 129 lbs.

Days	1	2	3	4	5	6	7
Total parasites	28,700	7,753	1,153	1,000	150	200	140
Maximum temp.	92	60	76	50	36	36	26
Methylene blue, grs.	—	—	—	12	12	12	12
Total leucocytes	—	65	50	56	71	47	48
Hb. Tal., percentage	—	90	85	80	85	85	80
Ur. urob., mgs.	19	14	23	12	—	—	—
Faec. urob., mgs.	0	27	0	0	—	—	—

Case 5—*continued*

Days	8	9	10	11	12	13	14
Total parasites	200	64	96	32	78	0	26
Maximum temp.	20	28	32	24	18	16	26
Methylene blue, grs.	12	12	12	12	—	—	—
Total leucocytes	—	43	—	—	—	—	—
Hb. Tal., percentage	90	90	90	90	90	90	90
Ur. urob., mgs.	—	—	—	—	—	—	—
Faec. urob., mgs.	—	—	—	—	—	—	—
Days	15	16	—	—	—	—	—
Total parasites	6	30	—	—	—	—	—
Maximum temp.	28	22	—	—	—	—	—
Methylene blue, grs.	—	—	—	—	—	—	—
Total leucocytes	78	—	—	—	—	—	—
Hb. Tal., percentage	90	90	—	—	—	—	—
Ur. urob., mgs.	—	—	—	—	—	—	—
Faec. urob., mgs.	—	—	—	—	—	—	—

Case 6.—J. M., sailor, 19; Niger; 135 days; April, ?

Days	1	2	3	4	5	6	7
As. parasites	852	668	1,080	256	64	68	44
Sex. parasites	—	—	—	—	—	—	—
Maximum temp.	26	70	102	82	20	24	20
Q. bihydrochloride, grs. ...	0	0	0	30	30	30	30
Ur. urob., mgs.	—	18	1	115	15	0	15
Faec. urob., mgs.	—	—	—	286	403	130	83
Days	8	9	10	11	12	13	14
As. parasites	24	8	28	—	—	—	—
Sex. parasites	—	—	—	412	144	56	16
Maximum temp.	24	16	20	14	20	26	22
Q. bihydrochloride, grs. ...	30	30	30	30	30	30	30
Ur. urob., mgs.	0	0	—	—	—	—	?
Faec. urob., mgs.	100	—	—	—	—	—	20

Case 6—continued

Days	15	16	17	18	—	—	—
As. parasites	—	—	—	—	—	—	—
Sex. parasites	48	52	20	8	—	—	—
Maximum temp.	24	10	24	34	—	—	—
Q. bihydrochloride, grs. ...	30	30	30	30	—	—	—
Ur. urob., mgs.	—	—	—	—	—	—	—
Faec. urob., mgs.	—	—	—	—	—	—	—

Case 7.—W. M., fireman, 35; Niger; 90 days; May, 155 lbs.

Days	1	2	3	4	5	6	7
Total parasites	—	440	1,300	133	250	152	148
Maximum temp.	22	102	66	34	30	28	26
Q. hydrochloride, grs.	0	10	0	0	0	0	0
Total leucocytes	20	40	22	85	110	56	150
Hb. Tal., percentage	70	80	75	85	90	85	80
Faec. urob., mgs.	—	0	530	340	88	0	200

Days	8	9	10	11	12	13	14
Total parasites	28	96	100	130	80	124	228
Maximum temp.	26	24	34	24	26	30	28
Q. hydrochloride, grs.	0	0	0	0	0	0	0
Total leucocytes	215	151	59	62	230	149	110
Hb. Tal., percentage	85	85	—	80	90	90	—
Faec. urob., mgs.	130	0	0	0	70	50	—

Days	15	16	17	18	19	20	21
Total parasites	284	368	540	280	400	440	50
Maximum temp.	42	54	22	30	20	32	20
Q. hydrochloride, grs.	0	10	30	30	30	30	30
Total leucocytes	61	100	70	80	24	71	45
Hb. Tal., percentage	80	80	80	85	Sahli per cent.	80	80
Faec. urob., mgs.	—	—	—	—	—	—	—

Case 7—continued

Days	22	23	24	25	26	27	28
Total parasites	16	28	32	0	12	0	12
Maximum temp.	32	22	28	28	32	34	24
Q. hydrochloride, grs.	30	30	30	30	30	30	30
Total leucocytes	80	180	44	45	50	140	50
Hb. Tal., percentage	83	83	82	80	75	80	85
Faec. urob., mgs.	—	—	—	—	—	—	—
Days	29	30	31	32	33	34	35
Total parasites	0	0	0	0	0	0	0
Maximum temp.	30	36	30	38(?)	28	28	22
Q. hydrochloride, grs.	30	30	30	30	30	30	30
Total leucocytes	100	61	200	75	160	300	—
Hb. Tal., percentage	75	80	75	75	80	83	—
Faec. urob., mgs.	—	—	—	—	—	—	—
Days	36	37	38	—	—	—	—
Total parasites	0	0	0	—	—	—	—
Maximum temp.	30	52	—	—	—	—	—
Q. hydrochloride, grs.	30	30	30	—	—	—	—
Total leucocytes	320	185	160	—	—	—	—
Hb. Tal., percentage	95	100	100	—	—	—	—
Faec. urob., mgs.	—	—	—	—	—	—	—

Case 8.—P. D., fireman, 29; ?, 240 days; May, ?

Days	1	2	3	4	5	6	7
Total parasites	7,600	3,615	2,816	168	50	30	20
Maximum temp.	88	86	90	26	24	16	26
Q. hydrochloride, grs.	—	—	10	30	*et. seq.*	—	—
Total leucocytes	78	30	28	40	45	27	52
Hb. Sah., percentage	80	68	80	80	75	80	83
Ur. urob., mgs.	0	80	111	80	—	—	—
Faec. urob., mgs.	0	0	1,120	—	—	—	—

Case 8—*continued*

Days	8	9	10	11	12	13	14
Total parasites	16	0	0	8	0	4	0
Maximum temp.	30	24	26	28	20	20	24
Q. hydrochloride, grs.	—	—	—	—	—	—	—
Total leucocytes	56	115	300	400	240	200	125
Hb. Sah., percentage	70	78	75	75	77	70	80
Ur. urob., mgs.	—	—	—	—	—	—	—
Faec. urob., mgs.	—	—	—	—	—	—	—
Days	15	16	17	18	19	20	21
Total parasites	0	0	0	0	0	0	0
Maximum temp.	34	30	24	22	30	34	—
Q. hydrochloride, grs.	—	—	—	—	—	—	—
Total leucocytes	150	80	110	—	270	96	250
Hb. Sah., percentage	85	80	—	—	85	95	100
Ur. urob., mgs.	—	—	—	—	—	—	—
Faec. urob., mgs.	—	—	—	—	—	—	—

Case 9.—N. B., sailor, 24; Peru, 45 days; June, 161 lbs.

Days	1	2	3	4	5	6	7
Total parasites	15,400	9,600	12,180	9,600	50	40	0
Maximum temp.	103	32	97	34	36	22	25
Q. hydrochloride, grs.	—	—	20	30	*et seq.*	—	—
Mon. leucocytes...	15	75	8	72	116	36	65
Pol. leucocytes	41	40	30	42	25	40	65
Hb. Sah., percentage	95	90	90	95	90	85	85
Days	8	9	10	11	12	—	—
Total parasites	20	0	0	0	0	—	—
Maximum temp.	22	30	25	25	—	—	—
Q. hydrochloride, grs.	—	—	—	—	—	—	—
Mon. leucocytes...	35	45	48	105	48	—	—
Pol. leucocytes	35	32	75	132	35	—	—
Hb. Sah., percentage	80	90	90	85	90	—	—

C. *P. falciparum*—

Case 10.—A. M. ship engineer, 37; Kamerun, 140 days; January, 145 lbs.

Days	1	2	3	4	5	6	7
*As. parasites	1,000	10,000	14,700	8,650	2,600	0	—
Crescents	—	—	—	—	—	137	73
Maximum temp.	62	58	38	54	26	20	18
Q. sulph., grs.	—	—	—	20	20	20	20
Ur. urob., mgs.	—	100	75	75	155	45	20
Days	8	—	—	—	—	—	—
As. parasites	—	—	—	—	—	—	—
Crescents	13	—	—	—	—	—	—
Maximum temp.	18	—	—	—	—	—	—
Q. sulph., grs.	20	—	—	—	—	—	—
Ur. urob., mgs.	17	—	—	—	—	—	—

Case 11.—W. M., steward, 17; Niger; 190 days; January, 126 lbs.

Days	1	2	3	4	5	6	7
As. parasites	56,257	55,300	0	0	0	0	0
Crescents	—	—	4	16	0	15	0
Maximum temp.	90	68	44	18	20	16	17
Q. hydrochloride, grs.	30	30	30	30	30	30	30
Ur. urob., mgs.	75	1,730	500	250	237	120	9
Days	8	9	10	11	12	13	14
As. parasites	—	—	—	0	0	—	—
Crescents	(*et seq.* 5 days)			—	—	—	—
Maximum temp.	30	24	34	24	20	24	34
Q. hydrochloride, grs.	15	15	15	15	15	15	15
Ur. urob., mgs.	55	0	0	50	20	0	0

Case 12.—C. H., seaman, 24; Niger, 30 days; January, 112 lbs.

Days	1	2	3	4	5	6	7
As. parasites	24,600	47,000	45,250	2,250	0	0	0
Crescents	—	—	—	—	180	168	—
Maximum temp.	50	86	64	42	32	20	18
Q. sulph., grs.	—	—	20	20	20	20	10
Ur. urob., mgs.	128	133	125	143	51	50	90

* As. = Asexual.

Case 12—*continued*

Days	8	9	10	11	12	13	14
As. parasites	0	0	0	0	0	0	0
Crescents	48	72	25	26	34	37	9
Maximum temp.	20	18	20	18	20	18	12
Q. sulph., grs.	10	10	10	10	10	10	10
Ur. urob., mgs.	0	0	40	18	0	27	17
Days	15	16	17	—	—	—	—
As. parasites	—	—	—	—	—	—	—
Crescents	8	—	5	—	—	—	—
Maximum temp.	22	12	12	—	—	—	—
Q. sulph., grs.	—	10	10	—	—	—	—
Ur. urob., mgs.	0	0	—	—	—	—	—

Case 13.—G. N., negro seaman, 22; Congo; 30 days; January, 138 lbs.

Days	1	2	3	4	5	6	7
As. parasites	181,933	173,400	60,100	673	0	0	0
Crescents	0	0	0	0	0	0	0
Maximum temp.	108	100	46	50	26	20	20
Q. hydrochloride	30	30	30	30	30	30	30
Ur. urob., mgs.	—	—	680	100	100	100	60
Days	8	9	—	—	—	—	—
As. parasites	0	0	—	—	—	—	—
Crescents	0	0	—	—	—	—	—
Maximum temp.	20	20	—	—	—	—	—
Q. hydrochloride	15	—	—	—	—	—	—
Ur. urob., mgs.	20	112	0 *et. seq.* (14 days)				

Case 14.—J. M., sailor, 31; Gold Coast; 56 days; January, 158 lbs.

Days	1	2	3	4	5	6	7
As. parasites	28,100	30,050	410	0	0	0	100
Crescents	—	—	—	565	1,190	2,000	1,200
Maximum temp.	76	30	44	14	20	16	18
Q. bihydrochloride, grs. ...	10	20	0	10	0	—	—
Total leucocytes	78	87	81	56	62	75	50
Ur. urob., mgs.	0	4	0	20	24	27	39

Case 14—*continued*

Days	8	9	10	11	12	13	14
As. parasites	0	0	0	0	0	0	0
Crescents	1,500	1,300	1,090	1,500	1,108	1,130	740
Maximum temp.	20	14	14	12	18	10	—
Q. bihydrochloride, grs. ...	—	—	—	—	—	—	—
Total leucocytes	56	81	80	56	59	62	62
Ur. urob., mgs.	39	11	9	—	—	—	—
Days	15	16	17	18	19	20	21
As. parasites	0	0	0	0	0	0	0
Crescents	1,022	691	336	422	—	162	95
Maximum temp.	—	—	—	—	—	—	—
Q. bihydrochloride, grs. ...	—	—	—	—	—	—	—
Total leucocytes	50	68	59	—	81	56	—
Ur. urob., mgs.	—	—	—	—	—	—	—

Case 15.—W. B., fireman, 27; Vera Cruz; 49 days; January, 126 lbs.

Days	1	2	3	4	5	6	7
As. parasites	0	—	—	—	—	—	—
Crescents	6,700	—	4,370	4,700	4,000	—	2,200
Maximum temp.	16	14	22	normal	—	—	—
Q. hydrochloride, grs.	30	30	30	30	15	*et seq.*	—
Total leucocytes	112	—	100	87	97	—	125
Ur. urob., mgs.	0	0	—	—	—	—	—
Days	8	9	10	11	12	—	—
As. parasites	—	—	—	—	—	—	—
Crescents	1,870	937	937	770	470	—	—
Maximum temp.	—	—	—	—	—	—	—
Q. hydrochloride, grs.	—	—	—	—	—	—	—
Total leucocytes	168	137	134	121	147	—	—
Ur. urob., mgs.	—	0	0	—	—	—	—

Case 16.—J. G., seaman, 17; W. Africa; 70 days; February, 126 lbs.

Days	1	2	3	4	5	6	7
As. parasites	0	—	—	—	—	—	—
Crescents	244	107	99	230	146	220	197
Maximum temp.	18	24	14 normal		—	—	—
Total leucocytes	75	81	93	112	122	137	115
Q. sulph., grs.	—	—	—	—	—	—	—
Days	8	9	10	11	12	13	14
As. parasites	—	—	—	—	—	—	—
Crescents	94	200	107	125	152	97	140
Maximum temp.	—	—	—	—	—	—	—
Total leucocytes	78	128	107	112	93	106	100
Q. sulph., grs.	—	—	—	20	20	10	10
Days	15	16	17	18	19	20	21
As. parasites	—	—	—	—	—	—	—
Crescents	86	60	47	65	44	51	32
Maximum temp.	—	—	—	—	—	—	—
Total leucocytes	122	100	93	131	134	137	—
Q. sulph., grs.	30	30	*et seq.*	—	—	—	—
Days	22	23	24	25	26	—	—
As. parasites	—	—	—	—	—	—	—
Crescents	8	10	20	10	6	—	—
Maximum temp.	—	—	—	—	—	—	—
Total leucocytes	—	—	—	—	—	—	—
Q. sulph., grs.	—	—	—	—	—	—	—

Case 17.—R. B., seaman, 19; Congo; 49 days; March, 134 lbs.

Days	1	2	3	4	5	6	7
As. parasites	5,250	380	2,837	336	288	184	60
Crescents	0	—	—	—	—	—	—
Maximum temp.	70	70	40	34	28	26	22
Q. hydrochloride	—	—	—	—	—	—	—
Total leucocytes	31	25	—	—	25	—	—
Hb. Tal., percentage	70	60	65	70	75	75	75
Ur. urob., mgs.	33	69	90	51	190	43	43
Faec. urob., mgs.	—	—	0	1,140	—	—	—

Case 17—continued

Days	8	9	10	11	12	13	14
As. parasites	36	55	12	1,750	360	0	8
Crescents	—	—	—	—	—	—	—
Maximum temp.	20	22	32	64	44	30	24
Q. hydrochloride	—	—	—	20	—	—	—
Total leucocytes	—	—	—	26	—	—	—
Hb. Tal., percentage	75	80	80	75	70	75	80
Ur. urob., mgs.	64	75	70	29	183	25	11
Faec. urob., mgs.	—	—	—	—	—	453	—

Days	15	16	17	18	19	20	21
As. parasites	0	12	0	0	28	60	1,325
Crescents	—	—	—	—	—	—	—
Maximum temp.	18	20	20	18	22	20	54
Q. hydrochloride	—	—	—	—	—	—	10
Total leucocytes	68	—	—	—	68	—	—
Hb. Tal., percentage	80	80	80	85	85	85	85
Ur. urob., mgs.	76	68	76	19	25	72	150
Faec. urob., mgs.	—	—	—	—	—	—	280

Days	22	23	24	25	26	27	28
As. parasites	1,250	0	8	0	0	0	0
Crescents	—	—	—	—	—	—	—
Maximum temp.	50	30	20	22	18	16	22
Q. hydrochloride	10	—	—	—	—	10	30
Total leucocytes	40	—	—	—	—	—	—
Hb. Tal., percentage	80	75	80	—	85	—	90
Ur. urob., mgs.	103	68	44	42	70	70	—
Faec. urob., mgs.	—	—	—	—	—	—	—

Case 17—*continued*

Days	29	30	31	32	33	34	35
As. parasites	0	0	—	—	—	—	—
Crescents	—	25	—	0	—	12	—
Maximum temp.	18	22	16	18	18	28	22
Q. hydrochloride	30	*et seq.*	—	—	—	—	—
Total leucocytes	125	—	—	—	—	—	—
Hb. Tal., percentage	—	90	—	85	—	90	—
Ur. urob., mgs.	—	45	0	—	—	—	—
Faec. urob., mgs.	—	—	—	—	—	—	—
Days	36	37	38	39	40	—	—
As. parasites	—	—	—	—	—	—	—
Crescents	0	—	—	12	—	—	—
Maximum temp.	26	30	28	26	24	—	—
Q. hydrochloride	—	—	—	—	—	—	—
Total leucocytes	—	—	—	—	—	—	—
Hb. Tal., percentage	90	—	90	—	90	—	—
Ur. urob., mgs.	—	—	—	—	—	—	—
Faec. urob., mgs.	—	—	—	—	—	—	—

Case 18.—A. C., steward, 29; Congo; 77 days; March, 100 lbs.

Days	1	2	3	4	5	6	7
As. parasites	32	—	0	—	24	—	128
Crescents	32	—	15	—	24	—	36
Maximum temp.	32	32	30	32	26	20	26
Q. hydrochloride	—	—	—	—	—	—	—
Ur. urob., mgs.	—	—	116	47	81	54	38
Days	8	9	10	11	12	13	14
As. parasites	—	26	—	80	64	—	388
Crescents	—	28	—	68	52	—	52
Maximum temp.	20	28	28	24	20	10	10
Q. hydrochloride	—	—	—	—	—	—	—
Ur. urob., mgs.	33	7	15	10	—	—	—

Case 18—*continued*

Days	15	16	17	18	19	20	21
As. parasites	—	1,860	20	744	60	0	0
Crescents	—	72	32	16	24	20	84
Maximum temp.	20	38	38	26	16	20	18
Q. hydrochloride	—	—	5	5	5	5	5
Ur. urob., mgs.	—	—	—	—	30	32	12
Days	22	23	24	25	26	27	28
As. parasites	16	0	0	0	0	0	0
Crescents	92	180	128	196	228	264	212
Maximum temp.	18	26	22	26	16	26	14
Q. hydrochloride	5	5	5	5	5	5	5
Ur. urob., mgs.	10	0	0	—	0	—	20
Days	29	30	31	32	33	34	35
As. parasites	0	30	32	0	0	—	—
Crescents	276	286	138	208	136	128	100
Maximum temp.	24	84	30	22	30	32	30
Q. hydrochloride	5	10	30	30	*et seq.*	—	—
Ur. urob., mgs.	0	0	—	—	—	—	—
Days	36	37	38	39	40	41	42
As. parasites	—	—	—	—	—	—	—
Crescents	50	50	—	48	—	16	—
Maximum temp.	20	42	22	24	—	—	—
Q. hydrochloride	—	—	—	—	—	—	—
Ur. urob., mgs.	—	—	—	—	—	—	—
Days	43	—	—	—	—	—	—
As. parasites	—	—	—	—	—	—	—
Crescents	8	—	—	—	—	—	—
Maximum temp.	—	—	—	—	—	—	—
Q. hydrochloride	—	—	—	—	—	—	—
Ur. urob., mgs.	—	—	—	—	—	—	—

Case 19.—H. R., sailor, 20; Niger; 40 days; March, 110 lbs.

Days	1	2	3	4	5	6	7
As. parasites	3,310	2,543	184	0	4	8	0
Crescents	None	—	—	—	—	—	—
Maximum temp.	76	75	32	32	22	16	20
Q. hydrochloride	—	20	—	—	—	—	—
Total leucocytes	37	31	—	—	—	51	—
Hb. Tal., percentage	60	50	65	70	70	75	80
Ur. urob., mgs.	67	0	132	144	170	115	39

Days	8	9	10	11	12	13	14
As. parasites	0	0	0	0	24	0	140
Crescents	—	—	—	—	—	—	—
Maximum temp.	28	24	24	32	24	42	32
Q. hydrochloride	—	—	—	—	—	—	—
Total leucocytes	—	—	—	94	—	—	—
Hb. Tal., percentage	80	80	85	85	80	80	85
Ur. urob., mgs.	108	116	38	40	42	0	38

Days	15	16	17	18	19	20	21
As. parasites	200	—	0	—	0	—	0
Crescents	—	—	—	—	—	—	—
Maximum temp.	54	36	26	20	20	22	18
Q. hydrochloride	10	30	30	*et seq.*	—	—	—
Total leucocytes	—	—	128	—	—	—	—
Hb. Tal., percentage	80	70	80	—	80	—	80
Ur. urob., mgs.	—	116	30	13	0	—	—

Case 20.—F. B., steward, 24; Niger; 40 days; March, 129 lbs.

Days	1	2	3	4	5	6	7
As. parasites	4,172	580	22	20	4	0	0
Crescents	0	0	0	0	0	4	12
Maximum temp.	90	56	70	40	24	22	20
Q. hydrochloride	10	0	20	—	—	—	—
Total leucocytes	—	—	—	37	—	—	60
Hb. Tal., percentage	60	55	60	75	75	80	75
Ur. urob., mgs.	0	273	182	162	81	54	29

Case 20—continued

Days	8	9	10	11	12	13	14
As. parasites	0	0	12	0	0	—	—
Crescents	8	16	12	—	0	—	—
Maximum temp.	26	28	24	24	26	20	26
Q. hydrochloride	—	—	—	—	—	—	—
Total leucocytes	—	—	100	—	—	—	—
Hb. Tal., percentage	80	80	80	—	85	80	—
Ur. urob., mgs.	32	40	26	0	0	70	70
Days	15	16	17	18	19	20	21
As. parasites	32	—	200	—	2,698	4,000	3,750
Crescents	0	—	4	—	0	0	4
Maximum temp.	22	22	26	46	74	70	76
Q. hydrochloride	—	—	—	—	—	10	30
Total leucocytes	150	—	—	—	90	46	—
Hb. Tal., percentage	85	—	80	—	83	83	S. per cent.
Ur. urob., mgs.	—	0	—	8	72	107	81
Days	22	23	24	25	26	27	28
As. parasites	1,560	104	0	—	0	—	320
Crescents	0	0	0	—	32	—	88
Maximum temp.	38	32	24	24	26	24	16
Q. hydrochloride	30	30	30	30	30	30	30
Total leucocytes	42	35	35	—	—	—	—
Hb. Tal., percentage	55	60	70	—	80	—	80
Ur. urob., mgs.	29	7	10	23	21	0	8
Days	29	30	31	32	33	34	35
As. parasites	—	0	—	0	—	0	—
Crescents	—	68	—	56	—	8	—
Maximum temp.	20	24	18	24	24	20	20
Q. hydrochloride	30	30	30	30	20	20	20
Total leucocytes	—	170	—	300	—	210	—
Hb. Tal., percentage	—	80	—	85	—	90	—
Ur. urob., mgs.	0	0	—	—	—	—	—

Case 20—*continued*

Days	36	37	—	—	—	—	—
As. parasites	—	0	—	—	—	—	—
Crescents	—	36	—	—	—	—	—
Maximum temp.	20	24	—	—	—	—	—
Q. hydrochloride	20	20	—	—	—	—	—
Total leucocytes	—	264	—	—	—	—	—
Hb. Tal., percentage	—	90	—	—	—	—	—
Ur. urob., mgs.	—	—	—	—	—	—	—

Case 21.—W. T., seaman, 50; Niger; 28 days; April, 158 lbs.

Days	1	2	3	4	5	6	7
As. parasites	36,000	25,300	16,000	280	0	0	300
Crescents	0	0	0	28	28	24	24
Maximum temp.	61	58	52	32	20	20	24
Q. bihydrochloride	—	—	20	30	30	30	—
Total leucocytes	—	25	—	43	—	—	—
Ur. urob. mgs.	—	10	48	1,300	273	54	40
Faec. urob., mgs.	—	—	0	0	30	4,100	?

Days	8	9	10	11	12	13	14
As. parasites	—	0	—	0	—	0	—
Crescents	—	16	—	4	—	8	—
Maximum temp.	24	34	26	26	22	26	24
Q. bihydrochloride	—	—	—	—	16	20	20
Total leucocytes	—	55	—	34	—	54	—
Ur. urob., mgs.	39	19	—	—	10	—	—
Faec. urob., mgs.	0	333	—	—	—	—	—

Days	15	16	17	18	19	20	—
As. parasites	—	—	0	—	—	0	—
Crescents	—	—	4	—	—	0	—
Maximum temp.	16	30	24	28	22	24	—
Q. bihydrochloride	20	20	10	10	10	10	—
Total leucocytes	—	—	75	—	—	119	—
Ur. urob., mgs.	—	—	—	—	—	—	—
Faec. urob., mgs.	—	—	—	—	153	—	—

Case 22.—C. G., sailor, 30; Niger; 40 days; April.

Days	1	2	3	4	5	6	7
As. parasites	7,920	—	20,250	44,800	920	30	50
Crescents	24	—	32	56	224	584	920
Maximum temp.	64	82	76	70	36	18	26
Q. bihydrochloride	—	10	30	30	30	30	30
Total leucocytes	—	—	—	130	200	80	50
Hb. Tal., percentage	—	85	80	82	80	80	86
Ur. urob., mgs.	0	0	1,500	0	50	70	34
Faec. urob., mgs.	1,730	780	0	1,670	0	?	116

Days	8	9	10	11	12	13	14
As. parasites	0	0	0	?	?	0	?
Crescents	1,128	1,180	1,384	1,288	1,240	948	892
Maximum temp.	24	24	18	26	14	24	18
Q. bihydrochloride	30	30	30	30	30	30	30
Total leucocytes	80	50	70	75	60	60	140
Hb. Tal., percentage	—	75	77	75	73	65	70
Ur. urob., mgs.	45	95	100	120	14	9	7
Faec. urob., mgs.	?	0	0	0	120	0	560

Days	15	16	17	18	19	20	21
As. parasites	0	0	0	0	0	—	—
Crescents	856	824	612	336	508	264	132
Maximum temp.	10	16	26	24	30	30	10
Q. bihydrochloride	30	30	30	10	10	20	—
Total leucocytes	100	78	60	180	90	140	92
Hb. Tal., percentage	75	80	85	—	85	—	85
Ur. urob., mgs.	23	38	18	0	12	0	0
Faec. urob., mgs.	220	386	113	0	?	0	—

Case 22—*continued*

Days	22	23	24	25	26	27	28
As. parasites	—	—	0	—	—	—	—
Crescents	190	120	100	40	32	—	20
Maximum temp.	10	16	14	22	20	10	10
Q. bihydrochloride	—	—	—	—	—	—	10
Total leucocytes	150	115	135	106	70	—	78
Hb. Tal., percentage	—	80	—	80	80	75	78
Ur. urob., mgs.	—	—	—	33	27	52	38
Faec. urob., mgs.	—	—	—	0	0	720	373

Days	29	30	31	32	33	34	—
As. parasites	—	—	—	—	—	—	—
Crescents	—	4	—	0	—	—	—
Maximum temp.	20	10	14	10	—	—	—
Q. bihydrochloride	10	10	10	10	—	—	—
Total leucocytes	—	100	—	160	—	—	—
Hb. Tal., percentage	84	85	82	82	—	—	—
Ur. urob., mgs.	10	0	—	—	—	—	—
Faec. urob., mgs.	0	0	?	0	0	10	—

Case. 23—T. C., sailor, 17; Niger; 21 days; April, 110 lbs.

Days	1	2	3	4	5	6	7
As. parasites	24,000	5,520	1,700	500	1,200	160	—
Crescents	—	—	—	—	—	160	—
Maximum temp.	98	90	32	30	28	14	14
Q. bihydrochloride	20	—	—	—	—	—	—
Total leucocytes	52	—	—	—	30	—	—
Hb. Tal., percentage	85	80	80	80	80	85	—

Days	8	9	10	11	12	13	14
As. parasites	28,500	22,600	1,600	340	0	0	0
Crescents	608	635	720	780	552	368	432
Maximum temp.	86	100	86	30	32	20	20
Q. bihydrochloride	tannate	10	20	30	30	30	30
Total leucocytes	—	48	—	—	40	52	—
Hb. Tal., percentage	75	75	75	73	65	70	—

Case 23—continued

Days	15	16	17	18	19	20	21
As. parasites	0	0	0	0	—	480	—
Crescents	500	464	468	468	—	344	—
Maximum temp.	28	20	32	20	30	28	24
Q. bihydrochloride	30	30	20	—	—	—	—
Total leucocytes	—	102	—	—	—	—	—
Hb. Tal., percentage	80	85	85	85	—	85	—

Days	22	23	24	25	26	27	28
As. parasites	20	—	10,948	11,360	1,600	1,584	1,000
Crescents	160	—	152	20	24	20	8
Maximum temp.	28	30	86	90	54	22	12
Q. bihydrochloride	—	—	—	30	30	*et seq.*	—
Total leucocytes	—	—	62	60	34	33	30
Hb. Tal., percentage	80	—	80	80	75	80	85

Days	29	30	31	32	33	34	35
As. parasites	0	0	0	0	100	0	0
Crescents	8	4	20	24	16	4	4
Maximum temp.	20	20	32	32	20	24	36
Q. bihydrochloride	—	—	—	—	—	—	—
Total leucocytes	—	48	80	130	98	110	85
Hb. Tal., percentage	85	82	82	85	85	85	85

Days	36	37	—	—	—	—	—
As. parasites	—	0	—	—	—	—	—
Crescents	—	0	—	—	—	—	—
Maximum temp.	30	20	—	—	—	—	—
Q. bihydrochloride	—	—	—	—	—	—	—
Total leucocytes	—	65	—	—	—	—	—
Hb. Tal., percentage	—	90	—	—	—	—	—

Case 24.—J. K., steward, 29; Niger; 28 days; April, 126 lbs.

Days	1	2	3	4	5	6	7
As. parasites	5,190	900	1,000	450	900	720	25,000
Crescents	0	0	20	16	88	168	540
Maximum temp.	52	38	26	32	24	52	24
Q. hydrochloride	—	—	—	—	—	—	10
Soamine, grs.	—	—	—	—	—	—	—
Total leucocytes	—	—	—	—	60	80	58
Hb. Tal., percentage	80	80	80	75	72	80	75
Ur. urob., mgs.	—	48	24	11	20	10	0

Days	8	9	10	11	12	13	14
As. parasites	1,000	9,500	3,900	600	—	—	—
Crescents	672	348	548	500	532	564	636
Maximum temp.	80	60	30	36	34	34	32
Q. hydrochloride	—	—	10	—	—	—	—
Soamine, grs.	10	—	8	—	—	8	—
Total leucocytes	36	45	70	50	70	140	160
Hb. Tal., percentage	70	75	75	70	75	80	80
Ur. urob., mgs.	0	—	—	—	—	—	—

Days	15	16	17	18	19	20	21
As. parasites	0	0	200	2,800	1,600	8,300	15,000
Crescents	784	572	520	630	496	367	212
Maximum temp.	24	26	38	40	44	54	54
Q. hydrochloride	—	—	—	—	—	10	30
Soamine, grs.	—	—	5	—	5	—	—
Total leucocytes	75	138	70	45	70	68	60
Hb. Tal., percentage	80	82	82	82	75	75	80
Ur. urob., mgs.	(faecal)	140	—	—	(faecal)	80	—

Days	22	23	24	25	26	27	28
As. parasites	5,600	200	1,000	1,100	0	0	0
Crescents	200	188	132	108	92	128	68
Maximum temp.	54	32	20	34	32	30	24
Q. hydrochloride	30	*et seq.*	—	—	—	—	—
Soamine, grs.	0	0	—	—	—	—	—
Total leucocytes	35	55	42	36	78	56	120
Hb. Tal., percentage	80	82	82	80	82	80	85
Ur. urob., mgs.	—	—	—	—	—	—	—

Case 24—*continued*

Days	29	30	31	32	33	34	35
As. parasites	—	0	—	0	—	0	—
Crescents	—	28	—	40	—	16	—
Maximum temp.	30	30	24	32	18	22	26
Q. hydrochloride	—	—	—	—	—	—	—
Soamine, grs	—	—	—	—	—	—	—
Total leucocytes	—	90	—	200	—	160	—
Hb. Tal., percentage	—	85	—	85	—	85	—
Ur. urob., mgs.	—	—	—	—	—	—	—
Days	36	37	38	39	—	—	—
As. parasites	0	—	0	0	—	—	—
Crescents	12	—	8	0	—	—	—
Maximum temp.	30	26	22	26	—	—	—
Q. hydrochloride	—	—	—	—	—	—	—
Soamine, grs.	—	—	—	—	—	—	—
Total leucocytes	115	—	200	195	—	—	—
Hb. Tal., percentage	—	—	90	90	—	—	—
Ur. urob., mgs.	—	—	—	—	—	—	—

Case 25.—K. K., sailor, 20; Gambia; 20 days; April, 154 lbs.

Days	1	2	3	4	5	6	7
As. parasites	3,000	4,000	3,956	14,076	13,392	18,000	2,100
Crescents	0	0	0	0	0	0	0
Maximum temp.	86	88	70	98	74	74	28
Q. bihydrochloride	—	—	—	10	20	30	30
Ur. urob., mgs.	76	30	10	—	—	—	—
Faec. urob., mgs.	120	—	—	—	—	—	509
Days	8	9	10	11	12	13	14
As. parasites	2,304	684	2,200	1,120	672	400	0
Crescents	0	0	0	0	0	0	0
Maximum temp.	36	30	32	28	26	26	22
Q. bihydrochloride	*et seq.*	—	—	—	—	—	—
Ur. urob., mgs.	—	—	—	—	—	—	—
Faec. urob., mgs.	—	—	—	—	—	—	—

Case 26.—A. M., sailor, 44; Niger; 20 days; May, 168 lbs.

Days	1	2	3	4	5	6	7
As. parasites	9,700	3,500	2,500	?	?	80	—
Crescents	0	*et seq.*	—	—	—	—	—
Maximum temp.	56	24	14	20	26	24	30
Q. hydrochloride	—	—	—	—	—	—	10
Total leucocytes	66	82	60	90	130	120	124
Hb. Tal., percentage	80	80	80	85	90	90	90
Ur. urob., mgs.	22	18	19	26	15	10	0
Days	8	9	10	11	12	13	14
As. parasites	400	2,000	300	0	0	0	0
Crescents	—	—	—	—	—	—	—
Maximum temp.	22	46	38	24	12	20	34
Q. hydrochloride	30	*et seq.*	—	—	—	—	—
Total leucocytes	160	155	176	168	150	120	—
Hb. Tal., percentage	90	80	88	88	84	90	—
Ur. urob., mgs.	10	—	—	—	—	—	—
Days	15	16	17	18	—	—	—
As. parasites	0	0	—	0	—	—	—
Crescents	—	—	—	—	—	—	—
Maximum temp.	16	10	20	24	—	—	—
Q. hydrochloride	...	—	—	—	—	—	—
Total leucocytes	200	—	178	—	—	—	—
Hb. Tal., percentage	90	—	90	—	—	—	—
Ur. urob., mgs.	—	—	—	—	—	—	—

Case 27.—S. P., trader, 30; W. Africa; Old Blackwater; May, ?

Days	1	2	3	4	5	6	7
As. parasites	1,104	—	—	—	1,056	600	400
Crescents	8	—	—	—	0	4	0
Maximum temp.	patient absent				14	12	30
Q. hydrochloride	0	*et seq.*	—	—	—	—	—
Total leucocytes	200	—	—	—	60	120	113
Hb. Tal., percentage	80	—	—	—	70	70	75

Case 28.—J. D., negro fireman, 25 (?); Sierra Leone; Niger; 42 days; June, 151 lbs.

Days	1	2	3	4	5	6	7
As. parasites	3,040	700	40	136	20	—	4
Crescents	0	*et seq.*	—	—	—	—	—
Maximum temp.	24	38	30	32	18	30	24
Q. hydrochloride	—	—	—	—	10	30	30
Total leucocytes	45	30	70	80	80	—	80
Hb. Tal., percentage	—	85	85	90	85	—	90
Ur. urob., mgs.	49	54	0	—	—	—	—
Faec. urob., mgs.	—	1,130	—	—	—	—	—
Days	8	—	—	—	—	—	—
As. parasites	0	—	—	—	—	—	—
Crescents	—	—	—	—	—	—	—
Maximum temp.	24	—	—	—	—	—	—
Q. hydrochloride	20	—	—	—	—	—	—
Total leucocytes	120	—	—	—	—	—	—
Hb. Tal., percentage	90	—	—	—	—	—	—
Ur. urob., mgs.	—	—	—	—	—	—	—
Faec. urob., mgs.	—	—	—	—	—	—	—

Case 29.—M. S., fireman, 38; Niger; 70 days; June, 149 lbs.

Days	1	2	3	4	5	6	7
As. parasites	5,300	6,500	3,800	0	0	0	0
Crescents	0	4	4	0	0	4	0
Maximum temp.	83	69	32	32	32	32	29
Q. hydrochloride	—	20	30	*et seq.*	—	—	—
Pol. leucocytes	21	21	22	15	20	28	41
Mon. leucocytes	12	35	64	18	45	43	100
Hb. Sal., percentage	60	55	65	65	60	60	65
Days	8	9	10	—	—	—	—
As. parasites	0	0	0	—	—	—	—
Crescents	0	0	0	—	—	—	—
Maximum temp.	32	32	25	—	—	—	—
Q. hydrochloride	—	—	—	—	—	—	—
Pol. leucocytes	78	45	20	—	—	—	—
Mon. leucocytes	128	45	30	—	—	—	—
Hb. Sal., percentage	70	65	70	—	—	—	—

Case 30.—J. W., sailor, 53; Niger; 15 days; June, 133 lbs.

Days	1	2	3	4	5	6	7
As. parasites	4,000	2,800	3	8	50	50	12
Crescents	0	0	0	0	0	20	80
Maximum temp.	79	54	22	32	16	18	18
Q. hydrochloride	20	0	*et seq.*	—	—	—	—
Pol. leucocytes	23	22	28	29	22	70	48
Mon. leucocytes...	15	85	62	62	30	70	56

Days	8	9	10	11	12	13	14
As. parasites	20	0	0	0	?	0	0
Crescents	120	152	120	116	88	80	72
Maximum temp.	22	22	25	22	29	34	29
Q. hydrochloride	—	—	—	—	—	—	—
Pol. leucocytes	78	35	35	72	42	82	90
Mon. leucocytes	123	60	28	52	50	78	63

Days	15	16	—	—	—	—	—
As. parasites	0	0	—	—	—	—	—
Crescents	68	56	—	—	—	—	—
Maximum temp.	32	25	—	—	—	—	—
Q. hydrochloride	20	20	—	—	—	—	—
Pol. leucocytes	142	63	—	—	—	—	—
Mon. leucocytes	98	73	—	—	—	—	—

Case 31.—J. R., sailor, 20; Niger; 90 days; June, 119 lbs.

Days	1	2	3	4	5	6	7
As. parasites	28,000	14,000	25,600	16,800	3,300	1,200	800
Crescents	0	*et seq.*	—	—	—	—	—
Maximum temp.	95	108	101	87	90	54	61
Methylene blue, grs.	—	4	*et seq.*	—	—	—	—
Pol. leucocytes	15	35	25	18	18	15	15
Mon. leucocytes	5	75	25	24	20	30	30
Hb. Tal., percentage	—	80	85	80	75	80	70

Case 31—*continued*

Days	8	9	10	11	12	—	—
As. parasites	0	0	0	0	0	—	—
Crescents	—	—	—	—	—	—	—
Maximum temp.	29	25	32	29	32	—	—
Methylene blue, grs.	—	—	—	—	—	—	—
Pol. leucocytes	15	17	20	65	92	—	—
Mon. leucocytes	38	39	36	135	88	—	—
Hb. Tal., percentage	65	70	75	75	75	—	—

Case 32.—F. H., sailor, 20; W. Africa; 150 days; July, 150 lbs.

Days	1	2	3	4	5	6	7
As. parasites	60	20	0	0	300	150	200
Crescents	244	100	108	52	80	104	60
Maximum temp.	25	22	25	36	29	40	22
Q. hydrochloride	0	*et seq.*	—	—	—	—	—
Pol. leucocytes	16	28	25	62	60	38	65
Mon. leucocytes...	25	20	62	125	102	48	132

Case 33.—Steward, 18; W. Africa; recently; July, 140 lbs.

Days	1	2	3	4	5	6	—
As. parasites	2,500	6,700	50	—	0	0	—
Crescents	0	*et seq.*	—	—	—	—	—
Maximum temp.	108 delirium	61 delirium	87 delirium	69 delirium	40	32	—
Q. hydrobromide	16	10	10	—	—	—	30 *et seq.*
Pol. leucocytes	55	35	28	—	35	32	—
Mon. leucocytes...	68	40	22	—	52	58	—
Hb. Tal., percentage	105	95	100	—	100	95	—

A CASE OF BLACKWATER FEVER FOLLOWED BY A PECULIAR RELAPSE WITHOUT HAEMOGLOBINURIA OR DETECTABLE *PLASMODIA*

BY

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This case was studied under the conditions and by the methods referred to in the previous paper, and is, we believe, the first case of blackwater fever which has occurred while daily estimations of the numbers of *Plasmodia* were being made by thick film methods. Though there is much evidence to connect blackwater fever with malaria, yet many authors doubt whether the typical fever generally associated with the haemoglobinuria is due entirely or at all to the parasites of malaria. Our case is of special interest because it throws considerable light on this question. It will be seen from the chart that the case had three separate pyrexial periods while under observation; that the first of these was an ordinary malarial attack; the second an ordinary blackwater attack (without detectable parasites); and the third a singular attack during which neither blackwater nor parasites were found—strongly suggesting, if not proving, that the later attacks were due to some cause other than the toxin of the *Plasmodia*, and that haemoglobinuria may be merely an occasional result of this unknown cause. A fourth attack occurred still later but, unfortunately, could not be studied by us.

The patient, A. E. B., male, aged 32, an English trader on the Gold Coast since 1903, stated that he had slight fever during his first year in West Africa, and numerous slight or severe attacks since then, but without any observed blackwater fever. He did not take much quinine until more recently, when (in February, 1910) he was given 30 grains daily for a fortnight, without its producing blackwater. He was admitted to the Royal Southern Hospital,

Liverpool, on the 2nd June, 1910, owing to the continuance of such attacks. His weight was 114 pounds (52 kilograms); he looked pale, thin and anaemic; his liver was enlarged half an inch below the costal margin, but his spleen, though enlarged, was scarcely palpable.

As shown on the chart, on the day of admission 2,600 asexual forms of *Plasmodium falciparum* were found per c.mm. of his blood, accompanied by the usual fever. On the following five days the same parasites numbered 80, 1,360, 880, 20, and 12 per c.mm., accompanied by fever on the 2nd, 3rd, and 4th days after admission. Quinine hydrochloride (10 grains) was given on the 4th day and 30 grains daily until the 10th day inclusive. No parasites were found on the 7th and 8th days, and no fever occurred on the 5th, 6th, 7th and 8th days. On the 7th and 8th days he was allowed out of hospital for a few hours in the afternoon, and on the latter day got heated with exercise, and ate ices.

On the 9th day he was attacked with high fever with rigors. On the 10th day haemoglobinuria commenced and the quinine was stopped. The attack was very serious. The haemoglobinuria cleared up on the evening of the 11th day, but the fever continued to a diminishing degree until the 18th day, though the temperature occasionally rose slightly above normal until the 37th day. There was then a break for four days, on the last of which he was again allowed out of hospital. On the 42nd day another severe pyrexial period commenced, lasting until the 54th day, but not accompanied by any haemoglobinuria. The patient now improved greatly and was discharged on the 63rd day; but returned again to hospital on the 76th day with fever (which, however, was not examined by us), but no blackwater. The parasites were carefully searched for (by D. T.), both in thick and thin films, on forty days up to the 49th day of the disease, after which, unfortunately, the examinations could not be continued. On not a single occasion since the 6th day of the disease could a single *Plasmodium* be found, though during the 9th, 10th and 11th days, that is, during the blackwater period, the blood was examined by both methods four times daily. No crescents were ever seen.

It should be noted that the haemoglobin of the blood, estimated (by D. T.) by Sahli's haemoglobinometer, began to fall on the 8th

day, the day before the fever of the 9th day commenced, and fell 10 per cent. daily during the next four days—the total fall being from 75 to 25 per cent. during seven days. It remained at the latter figure and then rose by about 5 per cent. daily for four days, and then more slowly. A slight fall interrupted the continuous rise on the 29th day, but on the 40th day the haemoglobin had reached 90 per cent. During the second relapse, occurring on the 42nd day, it underwent a total fall of only 12 per cent.

The red cells varied as follows:—

Days	11	12	14	18	20	21	22	25	29
Numbers in thousands	2,600	1,150	800	1,450	1,600	1,900	2,000	2,500	2,800

Regarding the urobilin (G. C. E. S.), there was only a very slight rise in the urinary excretion, reaching a maximum of 63 mgms. during the first febrile period. During this time two observations of the faecal excretion disclosed a total of 1,836 mgms. Commencing from the 10th day the faeces were examined continuously, and disclosed a urobilin excretion of 32;500 mgms. during six days, falling after this to the low figure of 100 mgms. per diem. During the blackwater period there was a marked degree of urinary urobilin, namely, 340 mgms. on the 10th day, 300 mgms. on the 11th day and 115 mgms. on the 12th day—after which it fell to the low level of 30 to 60 mgms. a day. The actual quantity of haemoglobin in the urine was estimated in each sample passed during the period of blackwater, and reached its height at 7 p.m. on the 10th day, and then fell gradually as follows:—

10TH DAY

Hours	10 a.m.	7 p.m.	11 p.m.
Quantity of urine	95 c.c.	90 c.c.	200 c.c.
Amount of Hbg. (expressed as red blood cells by volume) ...	Trace	4·5 c.c.	0·5 c.c.

11TH DAY

Hours	2 a.m.	9 a.m.	12 noon	7 p.m.	9 p.m.
Quantity of urine	250 c.c.	110	200	625	360 c.c.
Amount of Hbg. (expressed as red blood cells by volume)	0·625 c.c.	0·055 c.c.	0·33 c.c.	0·	1·98 c.c.

12TH DAY

Hour	3.30 a.m.
Quantity of urine	570 c.c.
Amount of Hbg. (expressed as red blood cells by volume)	0·

In all, the haemoglobin of 17 c.c. of normal blood or about 2·5 grams of haemoglobin was passed unaltered in the urine.

We should note that during the blackwater period and subsequent fever up to the 23rd day, chlorides were markedly diminished in the urine and the specific gravity was rather low.

During the febrile periods beginning on the 9th and on the 42nd days there was no scarcity of leucocytes such as occurred during the first febrile period, or such as occurs during ordinary malarial febrile periods; but the mononuclear percentage was high all through, as occurs during malarial fever and afterwards. Much bilious vomiting occurred on the 10th and 11th days, and unaltered bile pigment was present in the faeces up to the 15th day. The patient had the usual jaundiced appearance during the second febrile period.

Quinine, which had been stopped since the 10th day, was resumed in doses of one grain daily on the 35th day and two grains daily from the 41st to the 46th day, when it was stopped again on account of the second relapse. The urobilin was not estimated during the third pyrexial period.

On studying the case, we observe that the first pyrexial period from the 1st to the 4th day accorded exactly with our previous experiences with the thirty-three ordinary cases of malaria described in our first paper ('Enumerative Studies on Malaria'). The amount and type of fever, number of leucocytes, fall of haemoglobin, amount of urobilin excretion, and effect of quinine were precisely what we would have expected from the number of parasites counted; and the same thing may be said of the following apyrexial period. But the subsequent events differed markedly from our previous experiences. Although no parasites whatever were found in numbers of thick film preparations, yet the fever, fall of haemoglobin, and urobilin excretion in the later febrile periods were so severe that they would usually be associated with, say, 50,000 parasites or more per c.mm. The type of fever in these periods also differed from that of the first period and from that seen in our cases of malaria. During the second period (of the 9th day) the fever consisted of several severe paroxysms on each day, accompanied by sharp rigors and profuse sweats—all of these much more severe and continued than are usually seen in old cases of malaria, even with very many parasites. The third pyrexial period was very similar to the second one, except only

that it was not so severe, and that haemoglobinuria was entirely absent. Moreover, the whole comparatively fever-free period from the 18th to the 41st day, in which the temperature frequently exceeded normal, differed from ordinary malarial apyrexial periods, in which the temperature generally remains quite and even considerably below normal, and asexual parasites are often still to be found. Quinine certainly did not prevent the second and third pyrexial periods, although it was never followed by relapses in our cases of malaria; and the leucocytes did not show the characteristic diminution of malaria.

From these facts we are inclined to infer, almost with certainty, that the second and third pyrexial periods were not due to the toxin of the parasites of malaria, that is, to the usual cause of malarial attacks, but to some other cause. As shown in our previous paper, the fact that parasites were not found in no less than forty-three thick film specimens does not prove by any means that none were present in the whole body; but it proves that their numbers were much below the usual figure required to produce even slight fever. If, then, the later pyrexial periods were due to the small remainder of parasites, either the 'virulence' of these must have become suddenly and greatly exalted after the 9th day, or else the patient's resistance to their toxin must have become suddenly lowered. Neither of these appears probable, because the general type of the fever in the later periods differed so much from the usual malarial type, and because quinine had no reducing effect. If there is another cause, as seems to be highly probable from this case, it must exert a powerful haemolytic as well as pyrogenous effect. The third pyrexial period is of great interest, because it appears to establish a new fact, namely, that this peculiar fever, though generally associated with a great fall of haemoglobin and also with haemoglobinuria, need not necessarily be associated with either (the *Plasmodia* being at the same time undetectable). This proves also that mere haemoglobinuria by itself does not cause the fever.

We now content ourselves with merely recording the observed facts without discussing the possible causes of the fever. Further researches are required to ascertain whether similar phenomena occur in other cases. It has been often shown since Panse's* researches

* Otto Panse. Zeit. für Hygiene, 1902.

that the parasites tend to disappear at the onset of blackwater—but not, we think, so conclusively as in this case; nor do we know of relapses without either *Plasmodia* or haemoblobinuria having been previously noticed.

We should add (1) that no signs of any other parasites besides *Plasmodia* were seen; and (2) that the total elimination of urobilin from the 8th to the 15th day represents an amount of haemoglobin equal to that of *all* the patient's corpuscles, the 25 per cent. of haemoglobin still remaining being probably contained in young corpuscles generated during the period referred to. The relation between the numbers of circulating corpuscles and the haemoglobin percentage appears to confirm this estimate. As the asexual parasites cannot live outside the red corpuscles, this enormous destruction of the latter probably causes an equally great destruction of the former, which may explain Panse's law and the absence of parasites after the sixth day in our case; but it does not seem capable of explaining the fever. (3) The comparatively slight fall of haemoglobin during the third pyrexial period may be explicable on the supposition that the red corpuscles were then probably younger and more resistant. (4) The enlargement of the liver and the bilious vomiting might suggest derangement of the hepatic system, but they may also represent the hyperaemia of extreme activity. The fact that the liver eliminated, in its usual manner, such an enormous amount of blood pigment tends to confirm the latter hypothesis. (5) In conclusion, we would remark that haemoglobinuria seems to be merely a small overflow of the freed haemoglobin which the liver has not been able to deal with; and that some other cause other than the toxin of the *Plasmodia* seems to produce both the special haemolysis and the special fever associated with it.

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(June.) (L.B. age 26 Malig. Tertian Malaria; Blackw

Date.	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	1	2	3
Days.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32

Numbers of Leucocytes and Asexual Parasites per cu. mm. of Blood.

30000

20000

10000

Haemoglobin %

Allowed Out.

Allowed Out.

Red Cells 2,600000 per cu mm

" " 1,150000 " "

Red Cells 800000 per cu mm

Red Cells 1,450000 per cu mm

Red Cells 1,600000 per cu mm.

" " 1,900000 " "

Red Cells 2,000000 per cu mm

Red Cells 2,300000 per cu mm

Red Cells 2,800000 per cu mm

Total Leucocytes.

Asexual Parasites.

Quin. Hydrochlor. XXX grs. Daily.

(Temperature Fahrenheit)

105°
104°
103°
102°
101°
100°
99°
98°
97°
96°

ek water From.) 1. (July) (D. Thomson. Del.)

3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60

95%.
90%.
85%.
80%.
75%.
70%.
65%.
60%.
55%.
50%.
45%.
40%.
35%.
30%.
25%.

Percentage of Haemoglobin. (Sahli)

Patient Allowed Out.

Quin. Hyd. grain I Daily
" " " " " " "
" " " " " " "
" " " " " " "

ON HAEMOGLOBIN METABOLISM IN MALARIAL FEVER

BY

G. C. E. SIMPSON, B.A., B.Sc., F.R.C.S.

(*Received for publication* 15 *November*, 1910)*

I. INTRODUCTION

The following studies were carried out at the suggestion of Major Ross on some of the thirty-three cases of malaria which have occurred since January, 1910, at the Royal Southern Hospital, Liverpool, and which have already been reported upon by him and Dr. David Thomson in their paper on 'Enumerative Studies on Malarial Fever.'† The funds for the research were allotted to the Liverpool School of Tropical Medicine by the Advisory Committee for the Tropical Diseases Research Fund (Colonial Office). Much of the work was done in the laboratory of Professor Benjamin Moore, University of Liverpool, to whom we are indebted for advice and assistance.

The object of the research was to ascertain the fate of the haemoglobin, which is well known to be set free from the red corpuscles in malarial fever, and to measure its amount, in the ultimate hope that more light might thus be thrown on various problems connected with malaria, and especially with blackwater fever. The daily details of my findings are incorporated in the tables of the paper just mentioned, in order that they may be read in connection with the marked fall, recorded by the haemoglobinometer, in the haemoglobin of the circulating blood during the pyrexial periods.

II. PRELIMINARY

At first a careful search of the urine was made during and after the malarial paroxysms in eight patients. No trace of haemoglobin or of haemin or other immediate derivatives of haemoglobin was found, except in two cases, where a few red blood corpuscles were

* (Read in abstract before the Royal Society, Dec. 8, and reprinted from Proc. Roy. Soc., Series B., Vol. LXXXIII. p. 174.)

† Annals of Trop. Med. and Parasit., Vol. IV, No. 3, p. 267.

found associated with oxaluria. Hyaline and granular casts and oxalate crystals were present in the urine for a few days after the paroxysms in five of these cases (Nos. 10 to 14 inclusive). A greater measure of success was attained when attention was directed to the more remote products of haemoglobin breakdown; of these the iron-free pigment nucleus alone held out prospects of successful quantitative work, since the iron itself is known to be in a large measure retained in the body and the protein portion is merged in the general protein metabolism. Haemoglobin, whether arising by haemolysis in the body or experimentally introduced, is withdrawn from the circulation and broken down into its constituents by the liver, and the pigment moiety is excreted into the intestine as bile pigment. When amounts larger than about 0·3 gram of haemoglobin per kilo body weight are suddenly introduced into the circulating blood, some part may be excreted by the kidneys, and Barratt and Yorke* have shown that the haemoglobinuria varies with the degree of haemoglobinaemia.

The bile pigment leaves the body for the most part as urobilin in the urine and the faeces.

Since the relationship of haemoglobin and urobilin is known with fair accuracy, it is possible to calculate theoretically the amount of urobilin which would be yielded, if all the haemoglobin of a normal man could be transformed into that substance; or conversely what amount of haemoglobin must have been destroyed to give rise to a known excretion of urobilin. A man of 60 kilogrammes with a normal amount of red corpuscles, etc., would yield about 30 grams of urobilin from the haemoglobin (750 grams) of the circulating blood and about 20 grams more from the haemoglobin of his muscle and from pigment in the liver and bile passages.

III. URINARY UROBILIN

(*a*) The work of Hoppe-Seyler, Hildebrandt, and others has shown that urobilin is present only in traces in normal urines, but that it is increased in pyrexia, anaemias, hepatic disorders and internal haemorrhage. The excretion is from 0·01 to 0·1 gram per diem in the urine of healthy adults, and in pyrexias, etc., it rises as high as 0·5 gram per diem. Occasionally higher figures have been

* Annals of Trop. Med. and Parasit., Vol. III, No. 1, Section V.

recorded, 0·8 gram in scarlet fever, 2·02 gram in perityphlitis and 2 grams in malaria.

After trials of various methods, the method of quantitative determination adopted for the purpose of this work was to determine the dilution of the urine required to just render invisible the spectrum of urobilin, when examined by a spectroscope in a layer of 15 mm. thick. The spectroscope was standardised with solutions of purified urobilin, and the readings appear to be reasonably accurate except that, when only a low concentration of urobilin is present, they are too low. The spectroscopic results were checked in many instances by the more delicate fluorescence tests.

By this method I failed after repeated trials to find urobilin in any specimen of urine from healthy adults; amounts varying from 0·04 to 0·25 grams per diem were found in various pyrexial diseases and from 0·02 to 0·06 gram per diem in cases of anaemia, intermuscular haemorrhage and hepatic disease. In a case of Malta fever (temp. 104° F.) and in a case of acute suppurative hepatitis (probably dysenteric) (temp. 104° F.) 0·3 and 0·31 gram per diem were found respectively.

(*b*) In five cases of *simple tertian malaria* (*P. vivax*) the amount of urobilin found in the urine in the period of pyrexia and the days following was never greater than the average excretion in diseases such as pneumonia with an equal degree of pyrexia, and in Case 3, indeed, was scarcely perceptibly increased from the normal limits:—

Maximal excretion in twenty-four hours (Case 4) 0·19 gram.

Maximal total excretion in seven days after pyrexia (Case 4) 0·42 gram.

(*c*) *In malignant tertian malaria* (*P. falciparum*) a different picture is obtained; definite urobilinuria sets in very shortly after the onset of the pyrexia, reaches its height on the second or third day and then slowly diminishes; the normal level of excretion is reached in seven to ten days unless a fresh paroxysm intervenes. Sometimes the increased output continues for a longer period (*e.g.*, Case 20), and in old standing cases with chronic malarial anaemia there may be a continuous raised urobilin output even in the absence of pyrexia (Case 19); and in these cases marked haematoporphyrinuria was several times noted (Cases 17, 18, 19). (Haematoporphyrinuria was also noted on one occasion in Cases 20 and 23.)

Average excretions of urinary urobilin in malignant tertian malaria (Cases 10, 12, 17, 19, 20).

Maximum excretion in twenty-four hours (Cases 20 and 17) 0·2 to 0·30 grams.

Maximum total excretion in seven days (Cases 17 and 12) 0·6 to 0·7 gram.

The amount of urobilinuria (even if considered in comparison with body weight) bore no apparent relation (1) to the degree of pyrexia; (2) to the fall of the haemoglobinometer readings during the pyrexia; or (3) to the absolute numbers of parasites present in the blood.

(*d*) *Special high excretion in malignant tertian malaria.* In four cases the excretion of urobilin was so much higher that they will be mentioned separately and in more detail. In Cases 11, 13, and 23, the patients were young, and large numbers of parasites were present with correspondingly severe symptoms; in Case 21 the patient was older and, though the parasites were much less numerous and the pyrexia less marked, he was markedly prostrated by his attack.

I may mention that the urine in these cases became extremely dark on standing in daylight, after acidification, and that the band of urobilin remained visible in two instances until the urine was diluted to one hundred and fifty volumes. Cases 13 and 21 were constipated at the time the high-days' output took place.

I give in each instance the maximal twenty-four hours' output and the total excretion for seven days, and have also determined the percentage relation between these amounts and the normal circulating haemoglobin of the patients.

Number of Case	11	13	21	23
Weight in kilos.	60	54	72	50
Maximal 24 hours' excretion—				
As grams of Urobilin	2·31	0·9	1·73	2·0
As percentage of total Hbg.	8·1	3·5	5·0	9·5
Total 7 days' excretion—				
As grams of Urobilin	3·9	1·7	2·38	2·26
As percentage of total Hbg.	14·0	6·8	7·2	11·2
As corpuscles per mm.³ of blood	700,000	340,000	360,000	560,000
Fall in Haemoglobin as recorded by Haemoglobinometer	?	?	30 %	20 %
Number of parasites per mm.³ of blood	64,000	300,000	36,000	50,000

The figures are very high indeed, and equal, in some instances, any figure that I have found recorded by other observers; their special interest is that they show the destruction and elimination of a very large proportion of the haemoglobin of the circulating blood; I propose, however, to leave the question of urinary urobilin at this stage and proceed to consider the elimination by the bowel. As will be seen later urinary urobilin would appear to be simply a leakage—rarely an important one—from the other channel, and it has only been alluded to in consideration of the great attention that has been paid to it in the past, and since in the present instance, it was largely the promise held out by the results obtained in Cases 11 and 13 in the early days of January, that led to my persisting in further work along these lines.

Note.—Since writing this paper I have become acquainted with De Jonge's* results, he found urobilin in malarial urines in amounts of from 0·05 to 0·10 gram per diem, and this frequently rose as high as 1 gram and occasionally even to 4 grams. The difference may be accounted for by the fact that his cases, unlike mine, were frequently treated with quinine, which he found to increase the urobilin output. He found however lower outputs in malignant tertian malaria than in benign tertian, a result very different from mine.

IV. FAECAL UROBILIN

(*a*) It would be out of place in the present paper to discuss at any length the methods of extraction and estimation of urobilin from the faeces, but perhaps I may be pardoned for alluding to a few points of interest.

After considerable controversy it has been shown that urobilin of identical quantitative composition can, by various processes, be prepared from faecal and urinary sources. It would appear, however, that much of the urobilin in the faeces is present in modified forms (possibly in part in union with metallic salts) since it has a fluorescence and occasionally a spectroscopical appearance somewhat different from that of urinary urobilin; on one or two occasions I was very much puzzled by obtaining a solution much

* Geneesk. Tijdschr. v. Nederl.-Indie, Deel XLIV, p. 435, 1904.

more purple in colour than usual, and with a slightly altered band, and it was only after considerable expenditure of time that I discovered that part of the urobilin was present as its mercury compound owing to the administration of calomel.

Another variation which gave rise to great difficulty is that the urobilin, obtained by the extraction of the faeces with acidulated water, proves very refractory at different stages in the process of purification and separation, described by Garrod and Hopkins—indeed we have as yet failed to get a reasonably pure product from the faeces and so possibly the figures obtained may later require revision.

The urobilin was extracted by repeated shaking with large amounts of water, acidulated with dilute sulphuric acid, and the filtrates were fully exposed to daylight for some time, before being estimated by the same method as was adopted for urinary urobilin; this solvent has the advantage that it does not take up bile pigment or chlorophyll to any extent, and only on one occasion (later extracts of a case of blackwater fever) did I find any definite band other than that of urobilin, though other writers seem to have found such extra bands very frequently when using different solvents.

Another point of interest is that in such literature as I have discovered on faecal urobilin, the writers seem to convey the impression that it is present in small amounts and requires somewhat delicate tests for its identification. Only in McMunn's papers did I find descriptions of solutions comparable in colour and spectroscopic appearances to those with which I had become acquainted, and in which, as a general rule, a very high dilution was necessary before the general absorption was overcome; and the urobilin band was the only one ever distinguished save in the instance referred to in the last paragraph, when a band was also present embracing the D line, probably due to haematoporphyrin.

From Case 23, for example, nearly two litres of acidulated extract were obtained in which there were visible:—

1.	Acid Urobilin Band	to a dilution of		100	volumes.
2.	Mercury ,,	,,	...	120	,,
3.	Pink colour of mercury urobilin	,,	...	800	,,
4.	Fluorescence with Zinc Chloride	,,	...	300	,,

In another instance (blackwater fever case) from the same stool I obtained:—

4,000 c.c. of extract with Acid Urobilin band visible to 400 dilutions and Fluorescence (Zinc Acetate) to 1,600 dilutions.

7,400 c.c. of extract with Acid Urobilin band visible to 200 dilutions and Fluorescence (Zinc Acetate) to 1,000 dilutions.

3,000 c.c. of extract with Acid Urobilin band visible to 40 dilutions and Fluorescence (Zinc Acetate) to 600 dilutions.

In all 3,200 litres of fluid showing Urobilin band, or 15,600 litres of fluid showing clearly green Fluorescence with Zinc Acetate, in each case in test-tubes fifteen millimetres in diameter.

The only quantitative figures I have found in the literature for faecal urobilin are:—

0·021 to 0·029 gram per diem in cases of Chlorosis.
0·153 to 0·92 ,, ,, ,, Pernicious Anaemia.

My own results included the following:—

Stools of healthy adults on full diet	...	0·03 to 0·06 gram per diem.
Pneumonia (temp. 104°F., milk diet)	...	0·08 ,, ,,
Malta Fever (temp. 104°F., milk diet)	...	0·4 ,, ,,
Anaemic Girl (meat diet)	...	0·04 to 0·13 ,, ,,
Intramuscular Haemorrhage (meat diet)		0·12 to 0·13 ,, ,,
Amoebic Suppurative Hepatitis (temp. 103°)	...	0·12 to 0·13 ,, ,,
Chronic Malarial Anaemia ...	...	0·15 ,, ,,

(*b*) *Faecal urobilin in benign tertian malaria* (*P. vivax*). In three cases of this disease the excretion was found to be comparable to that found in other pyrexial diseases (in a fourth, Case 8, it was much higher, but was only taken on one day).

Case 6—Maximum day's excretion	...	0·49 gram.
Total excretion in 7 days	...	1·22 ,,
Control stool (13th day, meat diet)	...	0·02 ,,
Case 7—Maximum day's excretion	...	0·53 ,,
Total excretion in 7 days	...	1·30 ,,
Control stools (10th and 11th days, meat diet)	...	0·07 and 0·05 gram.
Case 8—Only one stool examined (4th day)	...	1·12 gram.

These excretions represent in Cases 6 and 7 about 4 per cent. of the patients' total circulating haemoglobin, the haemoglobinometer shows a much greater fall (20 per cent. in Case 7).

(*c*) *Faecal urobilin in malignant tertian malaria* (*P. falciparum*). Eight cases of disease have been examined for faecal urobilin, and in practically every instance a higher output was shown.

In five instances, only isolated stools shortly after the paroxysm were examined, and usually also a control stool, when the patient was again convalescent and on meat diet.

Case Number	Day of disease	Faecal Ub. (grams)	Faecal Ub. (per cent. of Hbg.)	Fall of Haem. (Haemoglobinometer)
17	4	1·13	4	20
	13 (relapse)	0·45	1·6	10
	21 (control)	0·28	1·0	—
24	20 (relapse)	0·8	2·4	5
	16 (control)	0·14	0·42	—
25	1	0·12	—	—
	7	0·51	—	—
28	2	1·13	3·5	5

In the two remaining cases the faecal excretion was followed throughout the attack, save for occasional unavoidable losses. The history of these cases (21 and 23) has already been alluded to in the section dealing with urinary urobilin.

In Case 23 the urobilin output during convalescence and on meat diet was from 0·01 to 0·02 gram per diem, and during his pyrexia this rose to as much as 1·73 grams.

In the first seven days after the onset of pyrexia his total urobilin output was 6·4 grams, representing no less than 25 per cent. of his total haemoglobin (of this amount 4·3 grams appeared in the faeces, 2·1 grams in the urine). His haemoglobinometer reading fell 20 per cent. and recovered 6 per cent. in the last two days of the week; the number of red blood cells destroyed (calculated from urobilin excretion) must have been about 1,250,000 per mm.^{3}, while the maximum number of parasites observed was only 50,000 per mm.^{3}.

In two subsequent relapses his urobilin output was very much lower (about 1·5 grams), but in the first of these especially a much more marked fall occurred, as shown by the haemoglobinometer, and the recovery was much slower, suggesting possibly that the reserves of haemoglobin precursors had become exhausted.

Case 21 had a rather higher output of urobilin during convalescence, amounting to 0·15 gram per diem, but after pyrexia it

reached the high figure of 4·1 grams (following three days' constipation). Some stools were unfortunately lost, but his total urobilin output (urinary and faecal) amounted to 6·8 grams, which represents 20 per cent. of his total normal haemoglobin.

The haemoglobinometer readings fell 25 per cent., of which 15 per cent. was regained within a week. His urobilin output corresponds to a destruction of 1,000,000 corpuscles per mm.^{3}, while the maximum number of parasites seen in the peripheral blood was 35,000 per mm.^{3}.

V. SUMMARY

There can therefore be little doubt that, in malignant tertian (*P. falciparum*) infection, the excretion of haemoglobin derivatives is much higher than in other diseases with a similar degree of pyrexia; this excretion is accompanied by, and probably depends on, a fall in the haemoglobin present in the circulating blood, and would seem to demonstrate that the fall in the haemoglobinometer readings represents an actual destruction of red blood cells, not merely an alteration in the relative proportions of corpuscles and plasma in the peripheral blood.

In Cases 21 and 23, respectively, thirty-five and twenty-five corpuscles at least must have been destroyed for every parasite present, and this suggests either that the corpuscles are disintegrated mechanically (or as part of a protective mechanism) by the efforts of the spores to enter them, or else that the mother-parasite contains some haemolytic substance which is liberated on sporulation.

Finally I would like to draw attention to the relationship between the elimination by the urinary and faecal channels in these cases, and, indeed, in health and in other diseases. Only exceptionally does the urinary output approach the level of the faecal output, and yet practically all the quantitative work that has so far been done on urobilin, has been confined to the small and comparatively unimportant channel.

Before concluding I would like to refer to the eighth case of malignant tertian infection mentioned in dealing with faecal urobilin, and which, owing to its peculiar interest, is dealt with in a separate paper by Major Ross, Dr. Thomson and myself (p. 307).

When admitted to the hospital he had a fairly severe attack of malignant tertian malaria, and in the week following its onset he

eliminated about 3 grams of urobilin, the large majority in the faeces; this is equivalent to 10 per cent. of his total circulating haemoglobin, and corresponds with the elimination in ordinary cases of this infection; (under normal circumstances on full diet his faecal urobilin is about 0·18 gram per diem).

A week later he had another attack of fever, accompanied by haemoglobinuria—the complex known as blackwater fever—and in the first seven days of this attack he excreted the enormous amount of 33 grams of urobilin. In spite of marked urobilinuria during the period of haemoglobinuria only 1·25 grams of urobilin appeared in all in the urine, and of the remainder nearly the whole (29·3 grams) was present in one stool, of which some particulars were given in the preliminary remarks on faecal urobilin (this stool contained also a considerable amount of unaltered bile) Owing to his collapsed and unconscious state, several stools were lost, but his next three recoverable stools contained in all only some 0·8 gram of urobilin, (though the presence of diarrhoea may have led to an unduly low yield).

As will be remembered, a man of his weight (60 kilos) contains potentially some 30 grams from the haemoglobin of his circulating blood and probably about 20 grams more from other sources, so this patient seems to have suffered an almost total destruction of his circulating blood. The haemoglobinometer reading fell to 25 per cent., and so apparently some of the destroyed haemoglobin was rapidly replaced from other sources. The haemoglobinuria itself only accounted for the haemoglobin of 20 c.c. of blood, less than 0·5 per cent. of the total.

I do not propose to allude further to this case, but would like to indicate one other possible point of interest. Barratt and Yorke* state that haemoglobinuria arises in man, if a grade of haemoglobinaemia is attained exceeding that obtained by the solution of the haemoglobin of 0·25 per cent. by volume of wet red cells in the blood plasma; (roughly speaking, the volumes of plasma and corpuscles are equal in human blood, and so on this scale total haemolysis represents 100 per cent).

We have seen that in Cases 21 and 23 from 20 to 25 per cent. of the corpuscles were destroyed, and in these cases the period of pyrexia

* *Loc. cit.*, Section III.

(in which it appears legitimate to assume the haemolysis took place), occupied about thirty-six hours. So the haemoglobin of about 0·6 per cent. of red corpuscles was discharged into the plasma in every hour, if the haemolysis was spread evenly over the period of the crisis; and as the liver would need to remove this amount every hour from the whole blood to prevent the appearance of haemoglobinuria, any slight failure of the mechanism either from hepatic congestion or from all the haemolysis occurring in a very short period of time might serve to set up haemoglobinuria.

CONCLUSIONS

1. The main excretory channel for the pigment portion of the blood is the alimentary tract, and urinary urobilin represents only a small overflow from this source; the main elimination is in the faeces, and this has been largely neglected by previous observers, in favour of the urinary overflow; which, however, would seem to be of importance only as an indication of the absorptive activity of the intestine and almost negligible for quantitative purposes.

2. That the fall in the haemoglobinometer readings in malaria appears to represent an actual destruction of red corpuscles and elimination of their pigment by the normal excretory mechanism.

3. The haemoglobin breakdown during the pyrexia due to malignant tertian (*P. falciparum*) infection appears to be greater than in other pyrexial diseases or than in benign malaria (*P. vivax*), and may be many times greater than would be accounted for by the number of corpuscles infected by the parasites.

4. That as regards their effect of causing haemolysis, the parasites appear to be of different degrees of virulence in different individuals, and even in different paroxysms in the same individual (relatively at least, since the patent's resistance must be considered).

5. Normally the ordinary channels of excretion are capable of dealing with the free haemoglobin and rapidly remove it from the blood stream, and a very severe strain (over 25 per cent. of the total circulatory haemoglobin) can be sustained without their failure and the consequent onset of haemoglobinuria.

SOME OBSERVATIONS ON A CASE OF SLEEPING SICKNESS: COAGULATION TIME OF BLOOD, ALBUMOSES, CHOLINE, CEREBRAL SECTIONS

BY

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INTRODUCTORY REMARKS

The observations recorded in this paper, though somewhat incomplete, are not without interest. They were made on a case of sleeping sickness in Professor Ross's clinic at the Royal Southern Hospital, Liverpool. This work was done in conjunction with other researches on the same case.*

Patient, W. A., male, age 26, was infected by the parasite of sleeping sickness in September, 1909, while he was in North East Rhodesia. Trypanosomes were observed in his peripheral blood in Africa on November 17, 1909. He died of lung and pleural complications (pneumonia and empyema) on June 29, 1910. The exact period of disease may be approximately calculated to be 300 days or thereabouts. The somnolent stage was not well pronounced. The parasite found in his blood showed a peculiar morphological feature in that some of the stout forms possessed a posterior nucleus, and has been named *T. rhodesiense* by Stephens and Fantham.† This parasite was of a marked virulence in the laboratory animals. Such in brief is the history and peculiarities of the case.

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* Ross and Thomson. Proc. Roy. Soc., 82 B, pp. 411-415.

† Stephens and Fantham. Proc. Roy. Soc., 83 B, pp. 28-33 (1910).

I. *Coagulation time of blood, by Wright's method at* 37°C. (*blood heat*).

In order to understand the relations between the coagulation, leucocytes, haemoglobin and the parasites, the observations are tabulated below. They were made within the last fortnight of the patient's illness.

	Normal (Man)	June 14	June 16	June 21	June 29
Coagulation time ...	3 to 6 minutes (4 mins. 30 secs. average)	12′ 0″	5′ 25″	5′ 0″	Died
Leucocytes per mm.^{3}	10,000	25,000	4,500	3,200	
Haemoglobin index (Sahli's scale) ...	100%	50%	70%	50%	
* Parasites per mm.^{3}	—	38	32	56	

The observations are too few to lead to any definite conclusions, but one gets a general impression that the coagulation time is passing from the negative to the positive phase as the leucocytosis is passing towards leucopenia. The number of the parasites and the nature of the haemoglobin index are subject to very little variation.

II. *Absence of trypanosomes from the cerebrospinal fluid and blood.*

Patient died June 29th at about 5 p.m. Post-mortem made June 30th at about 11 a.m.

(1) The cerebrospinal fluid was carefully collected in sterilized tubes. It was mixed with a trace of blood and lymph (oedema of the brain).

(2) Tubes were centrifugalised for fifteen minutes, the supernatant fluid was pipetted off, smears were made, both from the deposits and supernatant fluid, fixed in alcohol, stained according to the Romanowsky method. The result was negative.

(3) Smears were made from the blood, fixed in alcohol stained with Romanowsky. Result, negative.

* Counted by Dr. David Thomson.

III. *Presence of albumoses in the cerebrospinal fluid.*

(1) Acetic acid and saturated sodium chloride reactions—positive.*

(2) Biuret reaction—positive.

Besides it appeared from the methodical application of other tests that the albumose belonged to the class of primary proteose.

IV. *The blood was examined for the presence of albumoses. The result was negative.*

V. *Absence of albumoses in the urine.*

The urine during life was examined for albumose. The reaction was negative; later on, in the preserved specimen of urine albumose was again found to be absent.

Patient had a large collection of pus in the pleural cavity, consequently the possibility that the albumose was due to empyema was thus eliminated by the result of the blood and urine examinations.

So that although the cerebrospinal fluid was tinged with blood, the process of exclusion leads to the conclusion that albumose was present in the cerebrospinal fluid only. According to Byrom Bramwell† the presence of albumins, together with lymphocytosis in the cerebrospinal fluid, are supposed to be of early diagnostic value in the incipient stage of parasyphilitic lesions like tabes and general paralysis of the insane. The exact origin of albumose is doubtful; either it may be due to chronic meningo-encephalitis or to toxic products of protozoa or bacteria. Whatever the cause may be it is evident that the presence of albumose is not restricted to the parasyphilitic lesions only.

Its presence in the cerebrospinal fluid in the *incipient* stage of chronic meningo-encephalitis (*T. gambiense*) requires to be determined. But how far it may be of diagnostic value in the case of sleeping sickness is another question.

VI. *Presence of choline.*

Mott and Halliburton have shown that in general paralysis of the insane the marked degeneration that occurs in the brain is

* Boston, Clinical Diagnosis (1904), p. 216.

† Personal communication.

accompanied by passing of the products of degeneration into the cerebrospinal fluid, and of these nucleo-proteid and choline (decomposition product of lecithin) are those which can be most readily detected.

This is not peculiar to general paralysis of the insane, but choline can be detected in various other nerve degenerations.

The point of peculiar interest is that the pathological changes in general paralysis of the insane and sleeping sickness are more or less similar. The test used for choline was that of Mott and Halliburton, viz., the production of characteristic octohedral crystals of the platinum double salt from the alcoholic extracts of the cerebrospinal fluid. The result was positive.

Control tests were made with the 15 per cent. alcohol and absolute alcohol used in the original test, but these were negative.

VII. *Blood.*

With the above test it was found that choline was absent in the blood.

VIII. *Absence of choline in the blood of animals infected with the parasite of the case.*

The test applied was the modified method of Mott.* It consisted in the formation of the brownish-black rectangular plates of *choline periodide*.

The number of the trypanosomes per mm.^{3} at the time of the experiment and before death of the animals are given below.

	Trypanosomes at the time of the experiment	Trypanosomes before death
Rat 1.—Weight 182 grams	20,085 per mm.^{3}	126,000 per mm.^{3}
,, 2.—Weight 220 grams	56,320 ,,	100,400 ,,
Guinea-Pig 1.—952 grams	3,328 ,,	6,000 ,,
,, 2.—880 grams	4,416 ,,	30,720 ,,
Two control rats	0	0

* *Lancet*, July 9, 1910, p. 80.

Thus choline was absent in the blood practically at the height of the parasite infection.

According to Plimmer* the brains of rats that have been inoculated with the human trypanosomes have shown the peculiar perivascular infiltration. It is interesting to note from my observations that choline failed to enter into the blood, or in other words the products of nerve degeneration have not passed into the blood.

IX. *Effect of products of nerve degeneration in the cerebrospinal fluid (i.e., choline and nucleo-proteid) from the case of sleeping sickness—on the coagulation time of blood and the percentage of haemoglobin in the rat.*

	Before inoculation of cerebrospinal fluid intraperitoneally	After inoculation of the same intraperitoneally				
		1st day	3rd day	4th day	5th day	6th day
	min. sec. min. sec.	min. sec.	min. sec.	min. sec.	min. sec.	min. sec.
Coagulation time (By Wright's method at half blood heat)	4 25 to 4 30	3 15	7 5	6 40	6 55	10 46
Haemoglobin ... (Sahli)	120%	80%	55%	40%	60%	80%

We may not be able to draw any inferences from the smallness of this experiment, but one fact remains that the cerebrospinal fluid from the case of sleeping sickness contained a product which influenced the negative and the positive phase of coagulation, and so far as is known on the subject, it may be the nucleo-proteid.

X. *Sections of the brain tissue.*

The brain tissue was kindly lent to me by Dr. Stephens to whom my thanks are due.

The tissue was embedded in paraffin, sections were cut and stained with haematoxylin and eosin.

* Allbutt and Rolleston. System of Medicine, Vol. II, Part II, p. 215.

Microscopical appearances.

The perivascular spaces around the blood vessels in the substance of the brain were infiltrated with mononuclear leucocytes, chiefly lymphocytes. This change is typical of the sleeping sickness brain where the infection is due to *T. gambiense*. The infiltration was not enormous. I have applied a method of counting the mononuclears in the perivascular spaces. The sections cut were 8 μ in thickness.

Length of the vessel in section in μ	Breadth of the vessel in section in μ	Number of the mononuclears
100	22·8	34
68	68	48
100	68	72
100	100	56
100	100	56
68	68	45
136	68	40
100	85	45
100	85	45
136	100	116
100	85	35
136	100	118
100	85	95
100	85	68
100	85	55
100	85	40
100	85	40
100	85	40
136	100	150
68	68	40
100	34	50
100	34	48
Average 102	72·8	61 mononuclears

That is in a space of 102 μ × 72 μ × 8 μ there are 61 mononuclear leucocytes.

Some of the brain tissue was sent to Dr. Mott, F.R.S., by Professor Ross for his opinion. The report given by Dr. Mott appears below:—

Report on the Examination of Brain Tissue sent by Major R. Ross

Two small portions of brain embedded in paraffin were received; one was a small piece of cerebellum; the other was a small portion of white subcortical tissue, location not definable. Sections of 5μ were cut and stained by polychrome, Giemsa and Heidenhain haematoxylin methods.

All the sections examined showed a well-marked perivascular infiltration with lymphocytes and plasma cells, indistinguishable from that which I have seen in all cases of sleeping sickness. The membranes were absent in both pieces of tissue so I cannot say they were affected. Apparently there was evidence of glia hyperplasia and proliferation in the subcortical tissue. Inasmuch as subcortical and cerebellar tissues always show a more marked perivascular infiltration than the cortex in early cases it would have been more satisfactory if different regions of the cortex could have been examined. For unless the perivascular infiltration affects diffusely the cerebral cortex (seat of consciousness) one would not expect marked lethargy. From an examination of a large number of cases of sleeping sickness I have come to the conclusion that the subcortical structures are affected primarily and that there is a parallelism between the depth of the lethargy and the intensity and diffuseness of affection of the meninges and cortical vessels with neuroglia proliferation and changes in the nerve cells. A large number of the vessels show fibrin filaments indicative of a pneumonic condition.

SOME OBSERVATIONS ON MALARIA IN RELATION TO SPLENIC ENLARGEMENT AND THE TREATMENT OF THE CRESCENTIC STAGE

BY

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During the last two years (1908 and 1909) the city of Bombay was highly infected with malaria, the malignant tertian type being most common. This was most marked in 1908, and large numbers of cases were admitted to hospital. Several of these were in a moribund state, and the blood was found to contain typical rings and crescents in large numbers. In spite of such severe infection, it was noticed that many of them had absolutely *no fever*, and the principal sign of malarial infection, viz., *splenic enlargement, was also absent.*

The patients were in a semi-comatose stupid state, and could not be made to understand questions. In some cases parasites were found only in small numbers, and several slides had to be examined before a ring or a crescent could be detected. The crescents were looked for in several flat thick drops, which were fixed by being heated to about 70° C. for from fifteen to twenty minutes after drying them. This causes the serum to coagulate, and fix the leucocytes, blood platelets, malarial crescents and filarial embryos, if the latter are present. The haemoglobin does not coagulate at this temperature, and can be washed out by dipping the slide in distilled water. Tap water is not suitable if the Giemsa stain is to be used, as the salts in the water precipitate the stain. The slide is then stained with Giemsa's stain in the usual manner. This method is similar to the thick film one devised by Major Ross.

Various drugs were tried to study their effects on the duration of malarial crescents in the blood. It is well known that quinine has not much action on them, and this was seen to be the case in the patients under my care. In the cases under observation, although quinine was always administered in 15 grain doses at the least, there was very little reduction noticed.

Picric acid has long been recommended in malaria, but to my knowledge it is not stated what effects it is supposed to produce on these organisms. In a case of tertian malaria this acid was tried without the least benefit. Its study was taken up in cases of crescents. such of the cases as had the ring forms also present in the blood had the standard treatment with quinine; injections of the latter were also resorted to in severe cases. As control to the picric acid treatment some of the cases were treated only with quinine, either by the mouth alone or with injections as well; whilst others had quinine together with mercury perchloride, arsenic, salol and potassium iodide. The cases treated with picric acid were specially selected for the severity of infection with crescents.

18 cases had picric acid by mouth.
4 cases had injections of picrate of soda (3 c.c. daily of a 4 per cent. solution).
28 cases had quinine (with injections in six cases).
6 cases had quinine together with mercury, arsenic, etc.

The dose of picric acid was gr. 2 twice or thrice a day.

Enormous quantities of quinine (between 500 and 700 grains in different cases, as much as 915 grains having been used in one case) were administered both per os and hypodermically without any marked effect on the crescents, but on the whole under picric acid treatment the crescents seemed to disappear sooner than by any other treatment, as can be clearly seen from Table 'A.' During the time the patient is having the acid, the skin, the nails and the sclerae of the eyes show a yellow tinge as in jaundice, and the urine, too, presents a similar misleading appearance. But on adding an inorganic acid, e.g., hydrochloric or nitric acid, the colour gets paler and not deeper as in the case of urine containing bile. In Table 'A' is shown the number of days during which the crescents were found after quinine treatment alone, as compared with those

cases which had quinine and picric acid also, in the cases in which more or less complete observations were possible.

This table clearly shows that cases which received the picric acid treatment lost the crescents earlier than those which had no picric acid. The only case of picric acid treatment where the crescents continued to appear in the blood for sixty-two days was one of the most severe cases of malaria that I have seen. The patient, who was a deaf mute, was quite moribund for days together; while the analogous case without picric acid treatment from the start showed very few crescents—in fact, the ordinary smears did not show any.

The cases which lost their crescents within ten days must be regarded as cases that had probably come at the end of the crescent infection stage and thus lost them early.

Out of four cases in which crescents disappeared between ten and twenty days, three absconded, and hence they are put down as incomplete cases, while it is quite possible that the fourth case had come at the end of the crescentic infection; in fact, he was admitted for croupous pneumonia, and crescents were only found on examination of the blood as a routine method. It is not at all likely that the pneumococcic infection could produce destruction of the crescents, because the case in the simple quinine group that had crescents for sixty-two days was also suffering from the same disease at the time of admission in the hospital. In the quinine group which lost the crescents between twenty and thirty days, one case is marked incomplete for the same reason, viz., that he absconded with the crescents in the blood.

It must be borne in mind that all the cases that received the picric acid treatment were cases of specially severe infection, so that the fact that fourteen out of seventeen lost their crescents between ten and thirty days compares very favourably with the four out of seventeen of the quinine group which lost their crescents during the same period.

Again, on referring to Table 'B,' one finds that the death-rate was higher in the quinine treated cases, whether those had the spleen enlarged or not, as compared with cases which had the picric acid treatment also.

Of course the number of cases is not large, but one must bear in mind that crescent cases as a rule are not found in large numbers,

and the two years under consideration were exceptional in the fact that their number was far in excess of those I have seen in past years.

Plehn, at the British Medical Association Meeting in 1908, made the statement that the longest period that the crescents are found in the blood was six weeks; but in two of my cases they were found exactly for two months, and in one of them the last date cannot be determined, as the patient absconded from the hospital. Further, one has to consider the fact that we have no means of determining the date of the first appearance of the crescents in the blood, as all the patients were admitted in a very advanced state of the disease. Thus the total duration of the existence of these parasites must be more than two months.

Another statement by the same author concerns the diagnosis of such cases with crescents by increased urobilin in the urine. The use of an alcoholic solution of zinc acetate and Lugol's solution is described by him as the most delicate test. Both these points I was not able to confirm in my series of cases. Many of these crescents cases showed no increase of urobilin except in the very early stage, and generally at this stage the rings are still present, so that the urobilin is to be attributed rather to the blood destruction by the rings than to the presence of the crescents.

Of the two cases which showed crescents for two months, one had, from the start very few, and still they persisted for the time stated. This patient had only quinine treatment. The other had a very heavy infection, and when admitted was moribund, and so was put on picric acid treatment, but as she hardly ever swallowed her medicine, injections of picrate of soda had to be resorted to for a few days during the second month, and then the crescents soon disappeared.

The injections of picrate of soda were not found to produce any quicker results than the method of administration of picric acid by the mouth; however, in the case mentioned above it was necessary, as the patient would not take the medicines readily.

The method of preparing the solution of picrate of soda for injection is as follows:—

Add 4 grams of acid picric to about 75 c.c. of boiling distilled water, and to this add, drop by drop, a strong solution of sodium hydrate, taking the reaction at the same time, with a solution of di-methyl-amido-azo-benzol till the latter no longer turns red. (Do not stop when the dimethyl turns red and subsequently fades, but go on adding the sodium hydrate solution till no change of

colour occurs.) Confirm with phenol-phthalein to see that no change of colour occurs; this latter is necessary to ascertain whether the solution has become too alkaline or not. At this stage, most of the picric acid is dissolved up. Make up the solution to 100 c.c. with distilled water; 3 c.c. of this solution is put up in test-tubes and sterilised at 120° C. in the autoclave. As the water evaporates in these tubes crystals are deposited, which can be readily dissolved either by slightly warming the tube or adding a few drops of sterile distilled water. The injections are no more painful than ordinary injections of vaccine, and do not produce any marked reaction, nor is there any nodule or swelling noticed at the site of injection. Scarcely any pain is present after 24 hours, so that there is no difficulty in injecting about 3 c.c. of this solution on consecutive days.

It will be noticed from the Table 'B' showing the percentages of recovery and death in these cases, that the percentage of death was nearly double in cases not treated with picric acid as compared with those treated with the drug. Again a very interesting point noticed in all these observations was the fact that the mortality was higher in cases where the patient had no obviously enlarged spleen. In some of these cases where a post-mortem examination was obtained, the weight of the spleen was not much above the normal, and in one case it was found quite small and tough.

Cases of benign tertian fever are generally mild, and splenic enlargement is not noticeable in most; however, in my series of twenty-seven cases of a fairly severe type of this infection three cases ended fatally, and none of them showed any splenic enlargement. In the cases of malignant tertian infection, either alone or combined with benign tertian, the mortality was very high in cases where the spleen was not found to be obviously enlarged. Thus the splenic enlargement may be looked upon as a protective response on the part of the organism to the attack of the malarial parasite; and in a case where this response does not occur, it has a tendency to end fatally. Of course in mild cases the response is also slight, but when one considers cases of moderate or extreme severity, this fact is most evident.

This appears to be a natural consequence when one considers the fact that it is the spleen that is most concerned in the destruction of these parasites. The spleen may be called the battlefield and the burial ground of the malarial parasites. Thus the splenic response is, to my mind, a very important feature for purposes of prognosis.

There were some minor points noticed in the course of the investigation which may be mentioned here. Thus one comes across cases where quinine is not absorbed at all per os, and no amount of

administration by that method is of any avail. Thus in one of my cases of mixed infection, i.e., benign tertian and malignant tertian. I found that no evidence of an alkaloid could be obtained in the urine by the picric acid and gold chloride tests, although the patient was getting fifteen to thirty grains of quinine hydrochloride daily. Together with this I found that the attacks of fever continued in spite of the quinine by the mouth. Hypodermic injections of quinine had to be resorted to, and the urine at once showed the alkaloidal test. The temperature became normal, and continued to be normal for a few days. Another attack of fever, due probably to a fresh infection, occurred, and quinine was injected with equally good result. However, I had to persist injecting it about half a dozen times and ultimately this had no effect too; the urine at that time was found to be free from quinine, thus showing that the injected quinine was not being absorbed.

The tolerance of the malarial parasite to quinine is also possible, as in some cases the patient gets an attack showing parasites in his blood, although he may be taking ten to fifteen grains of quinine daily. At this stage it is found necessary to increase the amount of the drug.

In an interesting paper on malaria by Darling* it is stated that the number of gametes diminish under quinine administration. He cites only one case, No. 48987 (*vide* Table 'C') to show that no diminution occurred when quinine was withheld. However, one must bear in mind that the patient had non-sexual forms also present in his blood at the time when these observations were made; and as quinine was withheld there is a likelihood of fresh gametes being continually formed.

If one studies the table carefully, one can see that at first there is a diminution in the number of gametes, and only in the last count a sudden increase was noticed. This shows that gametes can disappear even when no quinine is administered, and, therefore, we have up to now no evidence to show that quinine has any effect in diminishing the number of gametes. It is unfortunate that the observation was not made in Case No. 53,937, which was free from

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* Annals of Trop. Medicine and Parasit., Vol. IV, No. 2, 25th July, 1910.

young asexual forms. Recently observations on these lines have been undertaken by me which will be published at a future date.

Recently a case of crescents with rings has been put on quinine gr. 30 per day till no rings could be detected. After that the quinine has been stopped, and blood smears and drops have been examined at short intervals to see whether the crescents can diminish without the administration of quinine. The annexed Table 'D' shows that there is a natural tendency for crescents to diminish even when no quinine is administered. The patient has no fever. Liver and spleen are both moderately enlarged. The patient has symptoms of dyspepsia, general debility and irritability of temper. This latter characteristic has been often noticed in crescent cases. The drops show a large and varying number of crescents, although when counted in smears one does not come across such marked differences. This shows that the counting of the smears is not a correct guide for the actual number of crescents in the blood. The diameter of each drop was between 8 and 9 mm., and the crescents were counted in these at an interval of a few days.*

TABLE A

No. of days that the crescents took to disappear from the blood when examined in drops	No. of cases treated with picric acid	No. of cases where no picric acid was given	Remarks
Less than 10 days	1	1	
Between 10 and 20 days ...	7	4	†3 incomplete
,, 20 ,, 30 ,, ...	7	4	†1 ,,
,, 30 ,, 40 ,, ...	1	7	1 died on 34th day
,, 40 ,, 50 ,, ...	0	0	
,, 50 ,, 60 ,, ...	0	0	
60 days and over ...	1‡	1	

* In Table D it will be noticed that the number of crescents in the drops on the first occasion was very great, and that on the third day there has been a sudden fall, which was kept up to a certain extent in subsequent counts also. Quinine administration was stopped on the 28th September, 1910, and since then none has been given. Is it likely that quinine actually retards the destruction of crescents by lowering phagocytic activity? This is a question worth considering in future observations.

† These cases absconded with crescents in their blood, and the results could not be completely ascertained.

‡ My notes on this case are :—Total quantity of quinine given, 915 grains. Total quantity of picric acid by mouth, 182 grains. Patients hardly ever swallowed these medicines. In spite of the quinine, rings were found in the blood a month after the commencement of treatment, this circumstance being accounted for by the drug not having been taken properly. Injections of picrate of soda were therefore commenced. Ten injections were given, one on every alternate day (in all 30 c.c. of a 4 per cent. solution, i.e., 18 grains of the acid were used during a period of 21 days from 29th December, 1909, to 19th January, 1910). Crescents disappeared in 29 days after the first injection, and they were present altogether for exactly two months.

TABLE B.—Showing the comparative mortality of Malaria cases with and without splenic enlargement, and also of crescent cases under picric acid as compared with ordinary quinine treatment.

Nature of cases	Total cases	Cured	Died	Cured with splenic enlargement	Cured without splenic enlargement	Died with splenic enlargement	Died without splenic enlargement	Remarks
(No Picric.) Malignant tertian with rings and crescents ...	34	25 (73·53%)	9 (26·47%)	19 (82·61%)*	4 (17·39%)*	2 (22·22%)	7 (77·78%)	* In 2 cases no record has been kept as regards splenic enlargement
(Picric Treatment.) Malignant tertian with rings and crescents	22	19 (86·37%)	3 (13·63%)	10 (55·56%)‡	8 (44·44%) ‡	1 (33·33%) †	2 (66·67%)	† Had Picric acid for 2 days only. ‡ In 1 case no record of splenic enlargement has been kept.
Malignant tertian with rings only	38	26 (68·40%)	12 (31·60%)	17 (65·40%)	9 (34·60%)	3 (25%)	9 (75%)	
Benign tertian	27	24 (88·89%)	3 (11·11%)	12 (50%)	12 (50%)	0 (0%)	3 (100%)	
Malignant tertian rings plus Benign tertian ...	6	4	2	1	3	1	1	
Quartan	3	2	1	1	1	0	1	

TABLE C.—Case No. 48,987. Darling's Paper

1908.....	November	November	November	November	December
Date	11	24	27	30	4
Crescents ...	16	27	12	8	20

TABLE D.—Case No. 621. Shewing the natural diminution in the number of crescents without any quinine treatment.

On 21st September, 1910, rings and crescents were detected in smears; on and after 29th September, 1910, when observations were commenced, no rings could be detected.

	SMEAR					DROPS		
Date	Poly-morphs	Eosino-philes	Large hyalines	Lym-phocytes	No. of crescents per 100 leuco-cytes	Number of crescents in each drop 8-9 mm. diameter		
1910								
29 September	72	1	5	22	5	149	269	381
30 ,,	69	0	4	27	3	—	—	—
1 October	69	2	3	26	3	18	40	65
2 ,,	70	1	5	24	3	—	—	—
3 ,,	58	0	5	37	3	—	—	—
4 ,,	76	0	7	17	1	—	—	—
5 ,,	79	3	2	26	1	—	—	—
6 ,,	66	2	6	26	0	12	25	29
7 ,,	65	1	4	30	0	—	—	—
9 ,,	67	1	4	18	1	—	—	—
12 ,,	60	3	8	29	1	2	10	0
15 ,,	54	11	12	23	0	1	2	0
20 ,,	62	5	7	26	0	0	0	0

ON THE PECULIAR MORPHOLOGY OF A TRYPANOSOME FROM A CASE OF SLEEPING SICKNESS AND THE POSSIBILITY OF ITS BEING A NEW SPECIES *(T. RHODESIENSE)**

BY

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AND

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PREFATORY NOTE

As already stated in a report to the Advisory Committee for the Tropical Diseases Research Fund, dated May 9, 1910, I noticed early in February, 1910, while examining in class work a stained specimen of rat's blood infected with what was supposed to be *T. gambiense*, a marked peculiarity in the morphology. This peculiarity was so striking that I doubted whether the trypanosome with which I was dealing was really *T. gambiense*. On making enquiries I was told that the strain was derived from a case of sleeping sickness then in Prof. Ross's clinic in the Royal Southern Hospital, Liverpool. To make certain that there was no error in this statement I myself infected a rat from the patient's blood. The same forms were, however, again encountered. After convincing myself that these forms were constantly present in infected rats, and that they were not shown by the rats infected with the old laboratory strain of *T. gambiense* maintained at the Runcorn Laboratory, I decided through pressure of work to ask Dr. Fantham (now working in the Liverpool School of Tropical Medicine, under funds allotted by the Advisory Committee for the Tropical Diseases

* Read before The Royal Society on Nov. 3, 1910, and reprinted from Proc. Roy. Soc., B. Vol. LXXXIII, p. 28, with addenda.

Research Fund) to be so good as to assist me in the description of the morphology of this trypanosome. The following paper is the outcome of our joint work.—[J. W. W. Stephens.]

HISTORY OF THE STRAIN

The trypanosomes used during this investigation were obtained from W.A., male, aged 26, a native of Northumberland, who was infected in North-East Rhodesia in September, 1909. It is necessary to set forth the itinerary of W.A. while in Africa, as he was never actually in an area infested with *Glossina palpalis*, so far as records are available, and indeed was never nearer (Kasama) than some eighty-six miles from such an area.

He first went to South Africa in July, 1904, living in Johannesburg till the end of 1906. He then went to Salisbury for two years. About the end of November, 1908, he left Salisbury for North-Eastern Rhodesia, with a view to prospecting for minerals. The party consisted of two Europeans and ten natives. On the journey northwards he passed through Fort Jameson, Landazi, and Chinsali to Kasama, where he arrived about the beginning of June, 1909. During this northward journey, W.A. passed through an area infested by *Glossina morsitans*. He stayed two months at Kasama, a place from which we have no records of any species of *Glossina*.* On the return journey he passed through Mpika (where *Glossina morsitans* occurs), Serenje (no records of *Glossina*), and Mzaza (where there is *Glossina morsitans*). He left Mzaza on September 10, and travelling along the Luangwa River he reached Feira on September 28. During this part of the journey he would pass through an area infested by *Glossina fusca*, between Mzaza and Hargreaves.

He first fell ill on September 20, but after a rest of two days continued his journey. A short stay was made at Feira, and then the return journey was continued through the Hartley district to Salisbury, where it was found that he and one native† were suffering from trypanosomiasis—parasites being found in his blood in Africa on November 17, 1909.

* Bagshawe, S.S. Bulletin, No. 18, June, 1910, p. 197.

† The infection of the native, at first reported, has since been stated to be a mistake.

The patient stated that he had never been near either Lake Tanganyika, Lake Mweru or the Luapula River, which are known to be infested by *Glossina palpalis*. He thought, himself, that he contracted trypanosomiasis while travelling along the Luangwa River, between Mzaza and Feira. The Luangwa Valley is heavily infested with *Glossina morsitans*, but *Glossina palpalis* has not yet been found along the course of this river.

Dr. Bagshawe's article, already noted, on 'The Transmission in nature of *Trypanosoma gambiense*,' should be consulted as to cases of sleeping sickness contracted in areas infested by *Glossina morsitans*.

We may note that the case of W.A. has been studied by R. Ross and D. Thomson,* who have found a regular periodical increase in the numbers of the trypanosomes in the peripheral blood of the patient from day to day.

It is also of interest to record that the Rhodesian strain of trypanosome from W.A. is somewhat more virulent to rats and guinea-pigs than the old laboratory strain of *T. gambiense*, a fact already confirmed by other workers in the laboratory. Further, this Rhodesian trypanosome is resistant to atoxyl.

MORPHOLOGICAL FEATURES

It may be stated at once that the peculiarity of this Rhodesian trypanosome is that among the stout or stumpy forms some have the *nucleus at the posterior (non-flagellar) end*.

As will be seen from the accompanying figures (Plate XXII, figs. 2-10), the position of this 'posterior' nucleus varies. Starting from the stumpy forms in which the nucleus is in the middle (fig. 1), we have all transitions (figs. 2-8) up to that in which the nucleus is actually terminal (fig. 9) and posterior to the blepharoplast (kinetonucleus).

We may meet here any objections that may be raised that the trypanosomes with posterior nucleus are due to distortion, dried films being used, because:—

1. We have never found them, though persistently looked for, in films from the same animals infected with the old laboratory

* Roy. Soc. Proc., B., 1910, Vol. LXXXII, pp. 411-415.

strain of *T. gambiense* (figs. 21-25), treated in the same way, *i.e.*, dried films;

2. Further, we have examined the trypanosomes under discussion, by *intra vitam* staining with methylene blue—by this method the posterior position of the nucleus can be seen; and finally,

3. We have fixed wet films with sublimate-alcohol and with osmic vapour respectively, and subsequently stained them with haematoxylin, and found the same forms.

Rats inoculated with the Rhodesian strain usually show a few long thin trypanosomes in the peripheral blood in about three days. The stumpy forms of trypanosomes with the nucleus posterior (figs. 2-10) appear about the fifth or sixth day, and from this time onwards somewhat increase in number up to the seventh to eleventh day. They then form about six per cent. of the trypanosomes present, but may decrease again, varying from day to day.*

These stumpy forms with posterior nucleus (as depicted in figs. 2-8) are 17μ to 21μ long and 2μ to 3μ broad. The nucleus often shows a karyosome, and when at the level of the blepharoplast is often kidney-shaped (fig. 8). There is a well marked blepharoplast (kinetonucleus) and a definite undulating membrane with a flagellar border which terminates in a very short free flagellum. The cytoplasm of these forms is granular, especially at the anterior (flagellar) end, where coarse granules are seen in life, which granules are found to be chromatoid in nature on staining (figs. 2, 3, 5, and 8). These cytoplasmic characters are very like those seen in ordinary stumpy forms of *T. gambiense* (fig. 25). Sometimes a stained line is clearly seen in the stout forms, joining the blepharoplast to the nucleus (fig. 10). We have little evidence as to the relationship of these forms with posterior nucleus to the stumpy forms with the nucleus in the ordinary (central) position (fig. 1), so content ourselves with pointing out that the existence of trypanosomes with posterior nuclei is quite characteristic of this Rhodesian strain, and is so marked a feature that we are always able in films to distinguish it from the old laboratory strain of *T. gambiense*.

These forms with the posterior nuclei are not confined to rats

* The infected rats live about a fortnight. After the posterior nuclear trypanosomes are once seen they can be found on every subsequent day till the death of the infected host.

(30 'passages'), but occur also in *Macacus rhesus* (observed by Dr. Yorke), rabbits, and guinea-pigs infected with this strain. We have been unable to detect these forms in the case of sleeping sickness itself, for the number of trypanosomes present in the blood was always extremely scanty, and in infected rats showing an equally small number of trypanosomes we have also never been able to find them.

A subsidiary but perhaps not unimportant point in the Rhodesian strain is that many of the ordinary long forms have an elongated posterior end, and may be termed 'snout' forms (figs. 13-17). The 'snout' forms occur more especially during the first half or two-thirds of the period of infection in rats. These elongate forms, while not absent in the old laboratory strain of *T. gambiense*, yet are certainly more numerous in the Rhodesian strain, and even by means of this feature we are able to distinguish the strains. 'Snout' forms were frequently seen in the blood of W.A. (figs. 14, 16), and in guinea-pigs. Bruce and Nabarro* have already noted these forms with elongate posterior ends.

Again, although forms with the blepharoplast terminal (figs. 18, 19) are found in the Rhodesian strain, they are less numerous than similar trypanosomes with terminal blepharoplast in the old laboratory strain.

We may also note in passing that pear-shaped (fig. 11) or rounded forms with little or no free flagellum are not uncommon in the Rhodesian strain.

SUMMARY AND CONCLUSIONS

1. We attach some importance to the fact that the patient (now dead) from whom this strain was derived was never, as far as careful enquiries could elicit, in a *Glossina palpalis* area, but had been in many *Glossina morsitans* areas, and very probably in a small *Glossina fusca* area.

2. These posterior nuclear forms (figs. 2-10) have not, as far as we are aware, been described either in the blood of sleeping sickness patients or in the blood of animals infected with *T. gambiense*. Seeing how many competent observers have worked

* Roy. Soc.: Reports of the Sleeping Sickness Commission, No. I, Pl. 2, Fig. 4.

with *T. gambiense*, this is a striking point and can hardly be an oversight.

3. We ourselves have been unable to find posterior nuclear forms, though constantly searched for, in the blood of rats infected with the old laboratory strain of *T. gambiense*.

4. Posterior nuclear forms exist also, as is well known, in *T. transvaaliense*, and by Lühe these are regarded as developmental stages of *T. theileri*. Whether this be so or not, the condition is not parallel to the one under discussion, for the differences in *T. theileri* are characteristic of all strains of this trypanosome, but the forms which we have described in this paper have, we believe, never been seen before in any strain of *T. gambiense* in man or animals.

5. We are aware that parasitic flagellates may exhibit morphological variations due to changes in metabolism or to differences in environment. However, this Rhodesian strain of trypanosome with posterior nucleus was seen in rats infected from the patient before he was treated with drugs. At first sight also it might appear that the posterior nucleus denoted merely approaching division or even assumption of the round form. However, we have seen division of these posterior nuclear forms (fig. 12). In any case the peculiar morphological feature exhibited by the Rhodesian trypanosome, in the possession of a posterior nucleus, has not been recorded before.

As to the meaning to be attached to these forms, we will consider the following main possibilities:—

(*a*) That we are dealing with a 'variety' or 'local race' of *T. gambiense* due to some change of environment. The possibility in this case exists of *T. gambiense* having been conveyed by a species of *Glossina* other than *Glossina palpalis*, which may account for the peculiar morphology we record; but it must be added that we have no knowledge of any other trypanosome undergoing similar changes owing to such a cause. That it is a 'laboratory' variation we think is out of the question, as all the known laboratory strains of *T. gambiense* are practically identical in morphology.

(*b*) That we are dealing with a new species of trypanosome also producing Sleeping Sickness in man. In support of this view there is firstly, the morphology, which considered alone is strong evidence,

since the Rhodesian trypanosome differs from *T. gambiense* more than, for instance, *T. brucei* does from *T. evansi*. Secondly, there is the history, taken in connection with the fact that, so far as we know, *T. gambiense* is conveyed solely by *Glossina palpalis*, but even should it prove that *Glossina palpalis* exists in the districts through which W.A. travelled, then all the more probable is it that we are dealing with a new species of trypanosome.

It seems to us, however, that it is difficult in the absence of further knowledge to discriminate with certainty between these views. Yet, on account of its peculiar morphological features, this Rhodesian trypanosome at least merits a distinct designation as *T. gambiense rhodesiense*, adopting the trinomial nomenclature. Our own view, however, is that we are dealing with a new species of human trypanosome for which we propose the name *Trypanosoma rhodesiense*.

ANIMAL REACTIONS OF *T. RHODESIENSE*

The following table has been compiled from the data obtained by Dr. Yorke. For full details his paper* should be consulted.

Animal	Duration of life in days		Animal	Duration of life in days	
	T. rhodesiense	*T. gambiense*		*T. rhodesiense*	*T. gambiense*
Monkey—					
M. rhesus ...	9	27	Guinea-pig ...	39	42
,, ...	11	91	,, ...	39	120A
,, ...	14	—	,, ...	41	134
			,, ...	52	160
			,, ...	76	167
C. callithricus ...	8	108	,, ...	82	—
,, ...	10	149			
			Dog	9	64
			,, ...	12	34A
C. ruber ...	14	—	,, ...	13	—
			,, ...	13	—
Rabbit	19	60	Goat	45	85
,, ...	19	109	,, ...	46	150A
,, ...	26	125	,, ...	55	—
,, ...	26	140			
,, ...	30	64A	Donkey	28	—
,, ...	32	90A	,, ...	54	—
,, ...	45	150A			
,, ...	32A	Neg.	Pony	38	—

— signifies no experiment. A signifies still alive.

* Annals Trop. Med. and Parasit., Vol. IV, No. 3, p. 367.

EXPLANATION OF PLATE XXII.

All figures drawn with Abbé camera lucida, using 2 mm. apochromatic objective and 12 compensating ocular (Zeiss). Magnification 1800 diameters approximately.

Figs. 1-20.—Rhodesian strain of human trypanosome. Figures drawn from parasites in the blood of rats, except where otherwise stated.

Fig. 1.—Stout form with nucleus median.

Figs. 2-8.—Stout and stumpy forms, each with posterior nucleus. The nucleus is seen gradually to become more posterior, till it lies behind the blepharoplast (fig. 9).

Fig. 10.—Posterior nuclear form with line connecting blepharoplast and nucleus.

Fig. 11.—Posterior nuclear form becoming rounded.

Fig. 12.—Posterior nuclear form showing division.

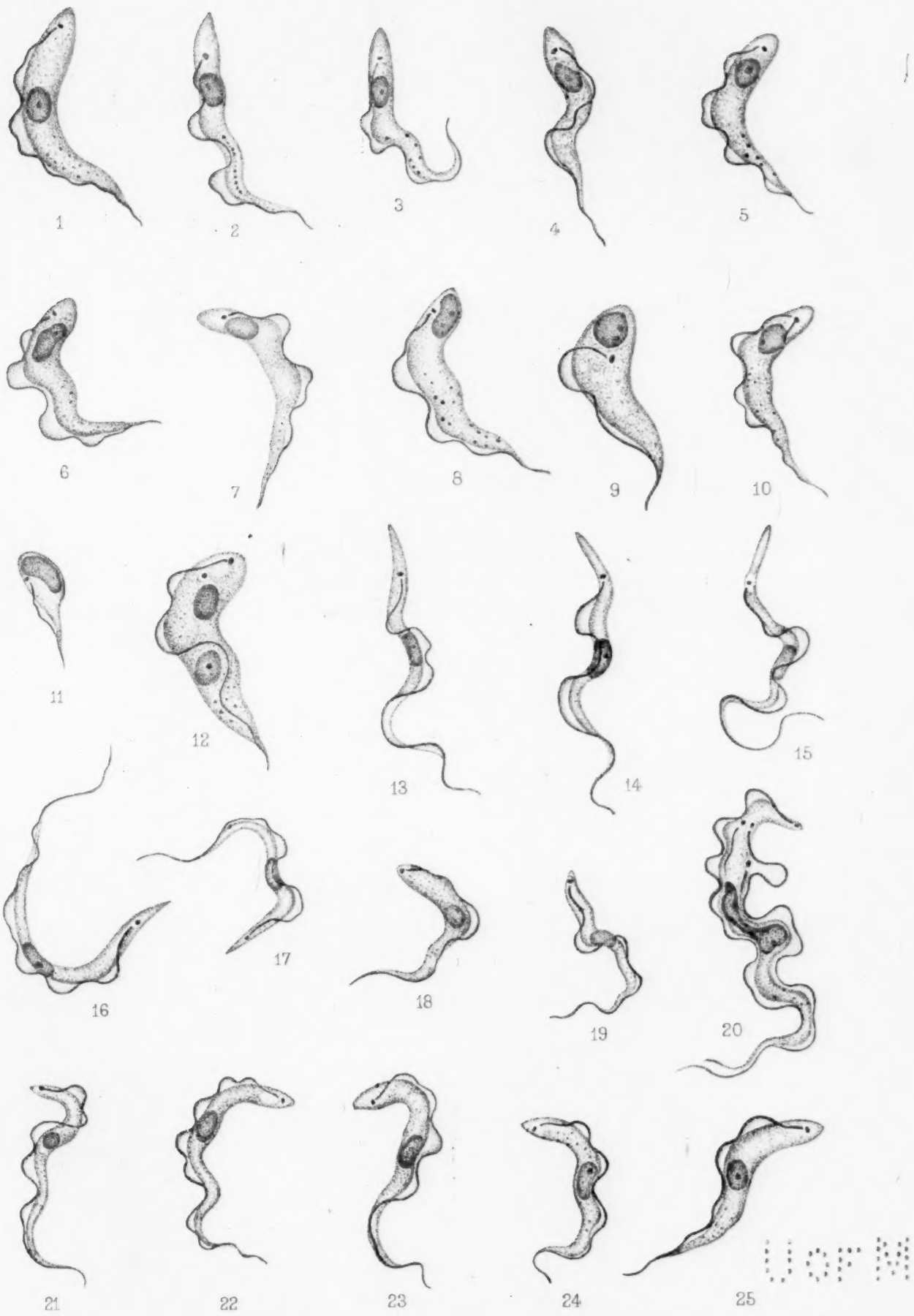
Figs. 13-17.—'Snout' forms. (Figs. 13, 15 from rat's blood, figs. 14, 16, 17 from man.)

Figs. 18, 19.—Forms with terminal blepharoplast.

Fig. 20.—Multiple division form, with four blepharoplasts and two nuclei. Such parasites are not uncommon in the Rhodesian strain.

Figs. 21-25.—Various trypanosomes drawn from the blood of rats infected with the old laboratory strain of *T. gambiense*.

PLATE XXII



H.B. Fantham del.

West, Newman lith.

NOW

ON THE PATHOGENICITY OF A TRYPANOSOME (*T. RHODESIENSE*, STEPHENS AND FANTHAM) FROM A CASE OF SLEEPING SICKNESS CONTRACTED IN RHODESIA

BY

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The question as to whether more than one trypanosome acts as a pathogenic agent in man is one which in the past has been much discussed.*

The findings of practically all workers are agreed in deciding that the trypanosomes infecting human beings in different parts of tropical Africa are identical.

Recently, however, doubt has been expressed regarding the identity of a human trypanosome of Rhodesia with that of *T. gambiense*†.

In December, 1909, a patient, W.A. (European), suffering from trypanosomiasis, was admitted into Major Ross's clinic in Liverpool.

So far as could be ascertained the patient had never been into any of the districts in which *G. palpalis* is known to occur. Exactly

* Castellani. 'Researches on the Etiology of Sleeping Sickness,' Jour. of Trop. Med. and Hyg., p. 167, 1903.

Thomas and Linton. 'A comparison of the animal reactions of the Trypanosomes of Uganda and Congo Free State Sleeping Sickness with those of *T. gambiense*,' Lancet, May 14, 1904.

Plimmer. 'Note on the effect produced on Rats by the trypanosomata of Gambian fever and Sleeping Sickness,' Proc. Roy. Soc., No. 504, p. 388, 1905.

Laveran. 'Sur trois virus de trypanosomiase humaine de provenances differentes,' Comptes Rendus de l'Acad. Sciences, p. 1065, 1906.

Gray and Tulloch. Reports of the S.S. Commission of the Royal Society, No. 8, p. 53.

Bentmann und Günther. 'Beiträge zur Kenntnis des *T. gambiense*,' Archiv für Schiffs- und Tropenhygiene, B. XI, Beiheft 2, 1907.

† Bevan and MacGregor. 'Note on the passage of a Human Trypanosome through domestic animals,' Journal of Comparative Pathology and Therapeutics, p. 160, 1910.

where the disease was contracted it is impossible to say; possibly, as the patient himself thought, it was in the Luangwa Valley.

Stephens and Fantham* have described certain morphological peculiarities, which the parasite exhibits in the blood of rats, rabbits and guinea-pigs infected with this strain. As these peculiarities have never been found in *T. gambiense*, the authors consider that this Rhodesian trypanosome is different from *T. gambiense*, and, in fact, a new species, for which they suggest the name *T. rhodesiense*.

In view of these morphological differences and of the fact that the disease was acquired in a district where *G. palpalis* has not yet been found, it appeared desirable to examine the pathogenicity of the parasite for animals of different species, with the object of comparing it with that of our laboratory strain of *T. gambiense* and with the results obtained by other workers with various strains of human trypanosomes.

Recent work has indicated that experiments upon the pathogenicity of a trypanosome must be conducted with certain precautions.

It was found by Thomas and Breinl† that a strain of *T. gambiense*, after passage through a baboon, had greatly increased in virulence. They state that rabbits, rats, cats, guinea-pigs and monkeys inoculated with the strain derived from the baboon developed the disease after short incubation periods, and that death occurred early.

Breinl and Nierenstein‡ found occasional variations in the virulence of *T. gambiense*. Rats infected from a monkey died in three to four days. After passage through guinea-pigs, however, the heightened virulence of the strain was lost and it killed rats again in the normal period, namely, forty to one hundred and twenty days.

Again, it is now generally recognised that long continued passage of a trypanosome through one species of animal tends to increase the virulence of the parasite for this species.

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* 'On the peculiar morphology of a trypanosome from a case of Sleeping Sickness and the possibility of its being a new species (*T. rhodesiense*),' Proc. Roy. Soc., Vol. LXXXIII, p. 28, 1910.

† 'Trypanosomes, Trypanosomiasis and Sleeping Sickness,' Liverpool School of Tropical Medicine, Memoir XVI, 1905.

‡ 'Bio-Chemical and Therapeutical Studies on Trypanosomiasis,' Annals of Trop. Med. and Parasit., Vol. III, p. 395, 1909.

The interesting observation of Schilling and Jaffé*, that mice, infected with a strain of Ngana, which had been preserved by passage through mice and subsequently treated with arsenophenylglycine, were temporarily immune to the mouse passage strain, but became infected on being inoculated with the same strain which had been kept up in guinea-pigs for two years, shows in what way the parasite can be modified by long passage through distinct animal species.

The work of Uhlenhuth, Hübner and Woithe† indicates that the quantity of infective blood injected may play an important rôle in determining the character of the resulting infection.

These authors, working with *T. equiperdum*, found that after inoculation of large quantities of infective blood, infection was delayed, and sometimes did not occur. Similar observations were made by Beck‡ with *T. gambiense*, who explains the fact on the ground that anti-bodies present in the infective blood act as trypanocides.

The strain which I have used in the following experiments was obtained by inoculating rats with the blood of the patient shortly after his arrival in England, before any medicinal treatment had been administered. The direct strain has been preserved by passage from rat to rat.

Before experiments on animals or other species were commenced, the strain had been passed through a series of twenty rats during a period of about six months.

For our laboratory strain of *T. gambiense* we are indebted to Professor Mesnil, who has kindly informed me that it was obtained by inoculating rats with the cerebrospinal fluid of a case of human trypanosomiasis from the French Congo, in 1905. Since then it has been kept up by passages through rats. Professor Mesnil adds that the strain has gradually become more virulent for rats, which it now kills on an average in twelve days.

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* 'Weitere chemotherapeutische Versuche bei Trypanosomenkrankheiten,' Archiv. für Schiffs- und Tropenhygiene, S. 525, 1909.

† 'Experimentelle Untersuchungen über Dourine mit besonderer Berücksichtigung der Atoxyl-behandlung,' Arb. a. d. Kaiserl. Gesundheitsamte, B. XXVII, S. 256, 1908.

‡ 'Experimentelle Beiträge zur Infektion mit *Trypanosoma gambiense* und zur Heilung der menschlichen Trypanosomiasis,' Arb. a. d. Kaiserl. Gesundheitsamte, S. 318, 1910.

In my experiments, the different animals were always inoculated with the blood of infected rats, so as to avoid any alteration in virulence comparable to that described by Thomas and Breinl, resulting from passage through a baboon.

With a view to avoiding any discrepancy arising from injection of widely varying numbers of trypanosomes, the injected blood was diluted with citrated saline solution, until it contained a definite number of parasites per cubic millimetre.

This was accomplished by diluting the blood of a heavily infected rat with many times its volume of citrated salt solution and then counting the trypanosomes in the diluted solution by means of the Thoma-Zeiss haemocytometer. The mixture was then further diluted with normal saline until one cubic millimetre contained 500, 1,000, 5,000 or 10,000 trypanosomes.

When cover-slip preparations of such solutions containing 1,000 trypanosomes per c.mm. were examined microscopically, it was found that there was approximately one parasite to a field (Zeiss, objective DD; eyepiece No. 4).

MONKEYS.

Rhodesian strain. Six experiments. The following species were used, *Macacus rhesus*, *Cercopithecus callitrichus* and *Cercopithecus ruber*. In all, the disease ran a very rapid course. Trypanosomes appeared in the blood after short incubation periods, and were subsequently always present—often in very large numbers—until death occurred. Posterior nuclear forms* of the parasite were found in every case except Monkey 6 (*Cercopithecus ruber*), where the parasites, although constantly present, were never numerous, the largest number found being ten to a field. With the exception of emaciation the animals presented no symptoms until within two or three days of death, when they lay at the bottom of their cages in a drowsy condition, ate little, if anything, and hardly responded to stimulation. The temperature never rose above

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* Forms in which the nucleus was either close to the blepharoplast or actually posterior to it.

104° F. in any of the animals, but usually fell considerably shortly before death. Incubation period, three to five days; average four days. Duration, eight to fourteen days; average eleven days.

TABLE I.—Monkeys infected with the *Rhodesian Strain*

Description of animal	Amount of virus injected subcutaneously or intraperitoneally	Day on which parasites were first seen in blood	Day on which death occurred	Remarks
1. *Macacus rhesus.* Weight 2210 g.	Intraperitoneally with 1 c.c. of diluted blood solution containing 5000 trypanosomes per c.mm.	5th day	14th day	Parasites always present in large numbers. Many posterior nuclear forms found. Weight at time of death, 1850 g.
2. *M. rhesus.* Weight, 1670 g.	Intraperitoneally. 1 c.c. of diluted blood solution containing 10,000 trypanosomes per c.mm.	3rd ,,	11th ,,	Parasites numerous on the sixth and seventh days. Posterior nuclear forms present. Subsequently the number of trypanosomes diminished. Weight at time of death, 1500 g.
3. *M. rhesus.* Weight, 5370 g.	Subcutaneously. 1 c.c. of diluted blood solution containing 500 trypanosomes per c.mm.	4th ,,	9th ,,	Parasites present in large numbers. Posterior nuclear forms found. Weight at time of death, 5000 g.
4. *Cercopithecus callitrichus* Weight, 2520 g.	Intraperitoneally. 1 c.c. of diluted blood solution containing 10,000 trypanosomes per c.mm.	4th ,,	10th ,,	Parasites present in considerable numbers from the sixth day until death. A few posterior nuclear forms seen. Weight at time of death, 2100 g.
5. *C. callitrichus.* Weight, 3160 g.	Intraperitoneally. 1 c.c. of diluted blood solution containing 5000 trypanosomes per c.mm.	4th ,,	8th ,,	Parasites numerous. Many posterior nuclear forms found. Weight at time of death, 2550 g.
6. *C. ruber.* Weight, 4150 g.	Intraperitoneally. 1 c.c. of diluted blood solution containing 500 trypanosomes per c.mm.	4th ,,	14th ,,	Parasites fairly numerous (10 to a field) on the ninth day; otherwise scanty. No posterior nuclear forms found. Weight at time of death, 3600 g.

T. gambiense. Four experiments. *Macacus rhesus* and *Cercopithecus callitrichus* were used. With this strain the disease ran a much more chronic course. Parasites were usually scanty, or absent from the peripheral circulation. Incubation period, three to twenty-one days; average twelve days. Duration, twenty-seven days to over four months.

TABLE 2.—Monkeys infected with *T. gambiense*

Description of animal	Amount of virus injected subcutaneously or intraperitoneally	Day on which parasites were first seen in blood	Day on which death occurred	Remarks
1. *Macacus rhesus.* Weight, 2490 g.	Intraperitoneally with 1 c.c. of diluted blood solution containing 5000 trypanosomes per c.mm.	3rd day	27th day	Parasites always scanty or absent. Weight at time of death, 2130 g.
2. *M. rhesus.* Weight, 3380 g.	,, ,,	5th ,,	91st ,,	Parasites scanty or absent generally, but fairly numerous during the last few days. Weight at time of death, 2600 g.
3. *Cercopithecus callitrichus* Weight, 2450 g.	,, ,,	21st ,,	149th ,,	Parasites as a rule scanty or absent. Animal very drowsy during last week. Weight at time of death, 1820 g.
4. *C. callitrichus.* Weight, 2730 g.	Intraperitoneally with 1 c.c. of diluted blood solution containing 10,000 trypanosomes per c.mm.	6th ,,	108th ,,	Parasites occasionally present in large numbers, but usually scanty or absent. Weight at time of death, 1750 g.

RABBITS.

Rhodesian strain. Eight experiments. In these animals the disease was fairly acute. Incubation period, from three to fourteen days; average eight days. Duration, nineteen to forty-five days; average twenty-eight days. Parasites were almost constantly present in the blood, although usually only in small numbers, except during the last few days, when they were generally present in considerable numbers. Posterior nuclear forms were usually to be found in small numbers on the occasions when the blood contained numerous parasites.

The symptoms presented were those usually found in rabbits suffering from trypanosomiasis, viz., blepharo-conjunctivitis, oedema of the head and ears, purulent discharge from the nose and eyes, loss of hair, emaciation and anaemia. Probably, owing to the shorter course of the disease, these symptoms were not so marked as in the rabbits infected with *T. gambiense*, where the disease is much more chronic. Two of the animals were very drowsy for a week before death (Nos. 5 and 6).

TABLE 3.—Rabbits infected with the *Rhodesian strain*

	Amount of virus injected subcutaneously or intraperitoneally	Day on which parasites were first seen in blood	Day on which death occurred	Remarks
1.	Intraperitoneally. 1 c.c. of diluted blood solution containing 1000 trypanosomes per c.mm.	9th day	45th day	Parasites scanty until the last few days, when they were fairly numerous, and when a few posterior nuclear forms were seen.
2.	Subcutaneously. 1 c.c. of diluted blood solution containing 10,000 trypanosomes per c.mm.	3rd ,,	32nd ,,	,, ,,
3.	Intraperitoneally. 1 c.c. of diluted blood solution containing 500 trypanosomes per c.mm.	7th ,,	26th ,,	,, ,,
4.	Intraperitoneally. 1 c.c. of diluted blood solution containing 1000 trypanosomes per c.mm.	5th ,,	26th ,,	,, ,,
5.	,, ,,	9th ,,	19th ,,	Parasites scanty, but constantly present until time of death, when they were fairly numerous (10 to a field). No posterior nuclear forms found. The animal was very lethargic during the last week.
6.	,, ,,	9th ,,	19th ,,	,, ,,
7.	Intraperitoneally. 1 c.c. of diluted blood solution containing 250 trypanosomes per c.mm.	14th ,,	—	Animal alive after 32 days. Parasites constantly present, but scanty. No posterior nuclear forms seen. Marked oedematous swelling of skin of head, loss of hair, ears thickened.
8.	Intraperitoneally. 1 c.c. of diluted blood solution containing 1:00 trypanosomes per c.mm.	8th ,,	30th ,,	Parasites only found in small numbers until last few days, when they were fairly plentiful (5 to a field). A few posterior nuclear forms found.

T. gambiense. Eight experiments. The disease was chronic. Parasites were only occasionally found in the peripheral blood, and always in very small numbers. Incubation, five to nineteen days; average twelve days. In one animal parasites were never found in the blood during the whole course of the disease. It presented the usual symptoms of infection, viz., oedema, loss of hair, muco-purulent discharge from nose and eyes, etc. Duration, sixty days to more than five months.

TABLE 4.—Rabbits infected with *T. gambiense*

Amount of virus injected subcutaneously or intraperitoneally	Day on which parasites were first seen in blood	Day on which death occurred	Remarks
1. Intraperitoneally. 1 c.c. of diluted blood solution containing 5000 trypanosomes per c.mm.	5th day	125th day	Parasites only occasionally found, and always scanty.
2. Intraperitoneally. 2 c.c. of diluted blood solution containing 10,000 trypanosomes per c.mm.	—	60th ,,	Parasites never found in blood. It presented the usual signs of disease.
3. Intraperitoneally. 1 c.c. of diluted blood solution containing 1000 trypanosomes per c.mm.	9th ,,	140th ,,	Parasites rarely found, and only in very small numbers.
4. ,, ,,	15th ,,	109th ,,	,, ,,
5. Intraperitoneally. 1 c.c. of diluted blood solution containing 10,000 trypanosomes per c.mm.	7th ,,	—	Animal alive after five months. It presents marked symptoms of disease, e.g., loss of hair, etc. Parasites rarely present in blood.
6. Intraperitoneally. 1 c.c. of diluted blood solution containing 1000 trypanosomes per c.mm.	19th ,,	—	Animal alive after three months. Parasites occasionally found in small numbers.
7. ,, ,,	15th ,,	—	Animal alive after 64 days. Parasites rarely found, and very scanty.
8. ,, ,,	—	—	Parasites have not yet been found in blood. Animal perfectly normal after 23 days.

GUINEA-PIGS.

Rhodesian strain. Six experiments. Parasites generally present in the blood, often in considerable numbers. Posterior nuclear forms were usually found when the parasites were numerous. The animals presented no symptoms, and the disease ran a fairly chronic course. Incubation period, three to fifteen days; average eight days. Duration, thirty-nine to eighty-two days; average fifty-five.

TABLE 5.—Guinea-pigs infected with the *Rhodesian strain*

	Amount of virus injected subcutaneously or intraperitoneally	Day on which parasites were first seen in blood	Day on which death occurred	Remarks
1.	Intraperitoneally. 1 c.c. of diluted blood solution containing 1000 trypanosomes per c.mm.	15th day	41st day	Parasites scanty except during the last week, when they were fairly numerous (15 to 20 to a field). Posterior nuclear forms found.
2.	Intraperitoneally. 1 c.c. of diluted blood solution containing 10,000 trypanosomes per c.mm.	3rd ,,	52nd ,,	,, ,,
3.	,, ,,	12th ,,	39th ,,	Parasites present in blood regularly, but in small numbers. No posterior nuclear forms seen.
4.	,, ,,	7th ,,	39th ,,	Parasites constantly present, generally in large numbers. Many posterior nuclear forms found.
5.	Intraperitoneally. 1 c.c. of diluted blood solution containing 500 trypanosomes per c.mm.	6th ,,	82nd ,,	,, ,,
6.	Intraperitoneally. 1 c.c. of diluted blood solution containing 10,000 trypanosomes per c.mm.	3rd ,,	76th ,,	Parasites always scanty until the last few days, when they were fairly numerous (10 to a field). Posterior nuclear forms seen.

T. gambiense. Five experiments. Parasites usually present only in very small numbers or absent. Disease very chronic. Incubation period, three to forty days; average fifteen days. Duration, forty-two days to five and a half months; average more than four months.

TABLE 6.—Guinea-pigs infected with *T. gambiense*

	Amount of virus injected subcutaneously or intraperitoneally	Day on which parasites were first seen in blood	Day on which death occurred	Remarks
1.	Intraperitoneally. 1 c.c. of diluted blood solution containing (?) trypanosomes per c.mm.	40th day	134th day	Parasites not found in blood (except on 40th day) until the last two days, when they were present in small numbers.
2.	,, ,,	3rd ,,	167th ,,	Parasites rarely found in small numbers.
3.	Intraperitoneally. 1 c.c. of diluted blood solution containing 10,000 trypanosomes per c.mm.	7th ,,	42nd ,,	Parasites regularly present, and sometimes in considerable numbers (20 to 30 to a field).
4.	,, ,,	13th ,,	—	Parasites only found on three occasions in very small numbers. Animal alive and well after four months.
5.	Intraperitoneally. 1 c.c. of diluted blood solution containing 1000 trypanosomes per c.mm.	12th ,,	160th ,,	Parasites rarely found in very small numbers.

DOGS.

Rhodesian strain. Four experiments. The trypanosome proved to be extremely virulent in dogs. In Dogs 1 and 2, parasites were present in the blood in large numbers, and many posterior nuclear forms were seen. Trypanosomes, although constantly present, were never very numerous in Dogs 3 and 4, and none of the above varieties were observed. The animals rapidly lost their weight. Incubation, four to five days; average four days. Duration, nine to thirteen days; average twelve days.

TABLE 7.—Dogs infected with the *Rhodesian strain*

Description of animal	Amount of virus injected subcutaneously or intraperitoneally	Day on which parasites were first seen in blood	Day on which death occurred	Remarks
1. Mongrel. Weight, 5100 g.	Intraperitoneally. 1 c.c. of diluted blood solution containing 1000 trypanosomes per c.mm.	4th day	9th day	Parasites present in large numbers. Posterior nuclear varieties seen. Weight at time of death, 4300 g.
2. Irish terrier. Weight, 12,050 g.	Intraperitoneally. 1 c.c. of diluted blood solution containing 250 trypanosomes per c.mm.	5th ,,	13th ,,	Parasites present in large numbers. Posterior nuclear forms seen. Weight at time of death, 10,460 g.
3. Collie Weight, 7950 g.	Intraperitoneally. 1 c.c. of diluted blood solution containing 500 trypanosomes per c.mm.	4th ,,	13th ,,	Parasites 10 to a field on the sixth to ninth days. Subsequently they were scanty. No posterior nuclear forms found. Weight at time of death, 7150 g.
4. Spaniel. Weight, 17,090 g.	,, ,,	4th ,,	12th ,,	Parasites scanty. No posterior nuclear forms seen. Weight at time of death, 15,020 g.

T. gambiense. Two experiments. In both animals the disease ran a chronic course. Parasites were, as a rule, only found in small numbers. Incubation period, twelve to sixteen days; average fourteen.

TABLE 8.—Dogs infected with *T. gambiense*

Description of animal	Amount of virus injected subcutaneously or intraperitoneally	Day on which parasites were first seen in blood	Day on which death occurred	Remarks
1. Irish terrier. Weight, 9500 g.	Intraperitoneally. 1 c.c. of diluted blood solution containing 500 trypanosomes per c.mm.	12th day	64th day	Parasites found fairly regularly in small numbers. On the last two days trypanosomes were present in large numbers (30 to a field).
2. Mongrel. Weight, 10,250 g.	Intraperitoneally. 1 c.c. of diluted blood solution containing 1000 trypanosomes per c.mm.	16th ,,	—	Parasites found fairly constantly, but usually in very small numbers. Animal still alive on 34th day.

GOATS.

Rhodesian strain. Three experiments. In these animals the disease ran a fairly rapid course. Parasites were not infrequently seen in the blood, but in very small numbers only, and the posterior nuclear variety was never found. The only symptoms observed were: loss of weight, oedematous swellings—accompanied by slight loss of hair—of the skin and subcutaneous tissues over the nasal bones and beneath the eyes, and interstitial keratitis. The temperature of Goat 1 rose to 107° F. on the seventh day after inoculation, but quickly returned to normal (103-4° F.). The other two animals never exhibited any rise of temperature. Incubation period, four to eleven days; average seven days. Duration, forty-five to fifty-five days; average eight days.

TABLE 9.—Goats infected with the *Rhodesian strain*

Description of animal	Amount of virus injected subcutaneously or intraperitoneally	Day on which parasites were first seen in blood	Day on which death occurred	Remarks
1. Medium ♀	Subcutaneously. 1 c.c. of diluted blood solution containing 1000 trypanosomes per c.mm.	6th day	55th day	Parasites scanty. No posterior nuclear forms seen.
2. Large ♀	Intraperitoneally. 1 c.c. of diluted blood solution containing 5000 trypanosomes per c.mm.	4th ,,	46th ,,	Parasites scanty. No posterior nuclear forms seen.
3. Large ♂	Subcutaneously. 1 c.c. of diluted blood solution containing 1000 trypanosomes per c.mm.	11th ,,	45th ,,	Parasites scanty and rarely found. No posterior nuclear forms seen.

T. gambiense. Two experiments. Both animals became infected, but the disease was exceedingly chronic. Parasites have never been found in the blood of Goat 1, but two rats, inoculated with its blood on the forty-fourth day, became infected after an incubation period of twelve days. The animal is still alive, more than five months after inoculation, and has presented no symptoms of disease. Rats inoculated with its blood, on the hundred and

fifty-second day, did not become infected. Parasites (1 to 15 fields) were found in the blood of Goat 2 on the nineteenth day after inoculation, but were never observed subsequently. On the seventy-second day slight swelling of the face was noticed. Beyond slight wasting no other symptom was exhibited. Death occurred on the eighty-fifth day.

TABLE 10.—Goats infected with *T. gambiense*

Description of animal	Amount of virus injected subcutaneously or intraperitoneally	Day on which parasites were first seen in blood	Day on which death occurred	Remarks
1. Large ♀	Subcutaneously. 1 c.c. of diluted blood solution containing 10,000 trypanosomes per c.mm.	—	—	Parasites never found in blood. Rats inoculated with its blood on the 44th day became infected, but not those inoculated on the 152nd day. Animal alive and well after more than five months.
2. Medium ♀	Intraperitoneally. 1 c.c. of diluted blood solution containing 5000 trypanosomes per c.mm.	19th day	85th day	Parasites only found once (on the 19th day) in very small numbers. Rats inoculated with its blood on the day of death became infected.

DONKEY AND HORSE.

Rhodesian strain. Two donkeys and a horse were easily infected.

Donkey 1. Inoculated subcutaneously with a small amount of the virus. Parasites first appeared in the circulation on the fifth day. From the sixth to the eighth day trypanosomes were present in fair numbers (3 to 10 to a field), and a few posterior nuclear forms were found. Subsequently parasites were constantly present in very small numbers until the last week when the number again increased, and the posterior nuclear variety were again found. Temperature remained constant (103-4° F.) throughout the disease. Beyond wasting and an oedematous condition of the skin and

subcutaneous tissues of the face, there were no symptoms, until the forty-seventh day, when it was noticed that the animal was very weak. The next day it was down and unable to rise. Death occurred on the fifty-fourth day.

TABLE 11.—Donkeys and Horse infected with the *Rhodesian strain*

Description of animal	Amount of virus injected subcutaneously or intraperitoneally	Day on which parasites were first seen in blood	Day on which death occurred	Remarks
1. Donkey ♀ Weight, 364 lbs,	Subcutaneously. 1 c.c. of diluted blood solution containing 10,000 trypanosomes per c.mm.	5th day	54th day	Parasites constantly present, but, as a rule, scanty, except on last three days, when they were fairly numerous (10 to 20 to a field). Posterior nuclear forms found. Weight a week before death, 290 lbs.
2. Donkey ♂ Weight, 324 lbs.	Subcutaneously. 1 c.c. of diluted blood solution containing 1000 trypanosomes per c.mm.	7th ,,	28th ,,	Parasites constantly present in small numbers. No posterior nuclear forms found. Weight five days before death, 280 lbs.
3. Pony ♂ Weight, 644 lbs.	Subcutaneously. 1 c.c. of diluted blood solution containing 10,000 trypanosomes per c.mm.	7th ,,	38th ,,	Parasites constantly present, but only in small numbers until within a few days of death, when 3 to 5 to a field were found. A few posterior nuclear forms were seen. Weight three days before death, 548 lbs.

Donkey 2. Inoculated subcutaneously. Incubation period seven days. Parasites were subsequently constantly present in the blood in small numbers. No posterior nuclear forms were seen. Temperature 103-104° F. The only signs of disease were progressive wasting and debility. Death took place on the twenty-eighth day.

A *Horse* inoculated subcutaneously with a small dose of the Rhodesian strain exhibited parasites in its blood on the seventh day. Trypanosomes were subsequently regularly found, there

being sometimes as many as five to a field, but, as a rule, they were scanty. A few posterior nuclear forms were found on the thirty-second day, at a time when the total number of parasites in the blood was about five to a field. The animal rapidly lost weight. Before infection it weighed 650 lbs., five days before death 548 lbs. The temperature remained constant, between 103° and 103·8° F., until a few hours before death, when it fell to 99° F. On the twenty-fourth day after inoculation the animal appeared ill for the first time. A few days later distinct swelling of the skin, subcutaneous tissues below the eyes and over the nasal bones was noticed. This condition was accompanied by some slight loss of hair and persisted until death. On the thirty-fifth day the animal was very ill. It had lost nearly 100 lbs. in weight, could hardly stand and refused food. The cornea of the left eye was hazy, and the conjunctiva slightly injected. The next day the animal was down and could not get up. Death occurred on the thirty-eighth day. At the time of death parasites were present in the blood in fair numbers (4-5 to a field).

Mice.

Rhodesian strain. Twenty experiments. The disease ran an acute course. The animals were inoculated intraperitoneally with $\frac{1}{4}$ c.c. of blood containing approximately 500 trypanosomes to a cubic millimetre. Parasites were usually present in fair numbers, and the posterior nuclear forms frequently found. Incubation period, two to five days; average three days. Duration, three to twenty days; average eleven days.

Rats.

Rhodesian strain. Forty-five experiments. The strain has been preserved by passage through these animals. In each case the rats were inoculated intraperitoneally with 1 c.c. of infective blood well diluted with citrated saline solution—a cover-slip preparation of which showed, as a rule, one to five trypanosomes to a field. The disease was fairly acute, and parasites were often present in the blood in considerable numbers. Posterior nuclear forms were usually found when the blood contained numerous parasites. Very

little alteration in virulence has been observed after a year's passage through these animals. The two rats infected directly from the patient died in six and twelve days, respectively. The average duration of the disease in the first ten passages was 17·5 days, and in the last ten, 20 days. Incubation period, one to seven days; average four days. Duration, six to forty-five days; average nineteen days.

T. gambiense. Fifty-four experiments. Incubation period, two to ten days; average four days. Duration, six to twenty-nine days; average fourteen days.

With a view to comparing the animal reactions of the *Rhodesian Trypanosome* with those of *T. gambiense*, the above results, and those obtained by other workers* with various strains of human trypanosomes, are summarised in Table 12.

It is evident that the Rhodesian trypanosome differs very markedly from *T. gambiense* in its pathogenicity for various species of animals. In monkeys (*Macacus rhesus*, *Cercopithecus callitrichus* and *Cercopithecus ruber*), dogs, rats and mice the disease runs a very acute course. Large numbers of trypanosomes were usually present in the blood. Rabbits, guinea-pigs and goats were easily infected, and died in a much shorter time than animals of the same species infected with *T. gambiense*, and parasites were much more constantly found in the blood and in greater numbers. Two donkeys and a horse quickly succumbed to the disease, trypanosomes being regularly observed in the peripheral circulation.

These results show that in so far as virulence is concerned the parasite is widely different from *T. gambiense*—in fact it more closely resembles *T. brucei* in this respect than *T. gambiense*—and support the view advanced by Stephens and Fantham, on morphological grounds, that we have in '*T. rhodesiense*' a new species.

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* Reports of the Sleeping Sickness Commission of the Royal Society.

Thomas and Linton. *Loc. cit.*

Laveran. *Loc. cit.*

Brumpt et Wurtz. 'Maladie du Sommeil expérimentale chez les Souris, Rats, Cobayes, &c.,' Comptes Rendus Soc. Biologie, T. LVI, p. 567, 1904.

Beck. 'Experimentelle Beiträge zur Infektion mit *Trypanosoma gambiense*,' Arbeiten a. d. Kaiserl. Gesundheitsamte, S. 318, 1910.

Bentmann und Günther. *Loc. cit.*

TABLE 12.—Summary of the results obtained in various animals with the *Rhodesian strain* and *T. gambiense* respectively, and those of other workers with various strains of *T. gambiense*

	Rhodesian strain		*T. gambiense*		Results Obtained by Various Workers with Different Strains of Human Trypanosomes											
					Royal Society Commissioners in Uganda		Thomas and Linton		Laveran		Brumpt and Wurtz		Beck		Bentmann and Günther	
	Incubation	Duration	Incubation	Duration	Incubation	Duration	Incubation	Duration	Incubation	Duration	Incubation	Duration	Incubation	Duration	Incubation	Duration
Rat	4 days	19 days	4 days	14 days	20 days	3–8 + mths.	8 days	45–388 days	G.* 13 days U. 11 ,, K. —	62 days 77 ,, 81 ,,	3–4 days	4–5 months	3–5 days	3–4 mths.	U. 4·9 days K. 12·5 ,,	69 days 81 ,,
Mouse	3 ,,	11 ,,	—	—	—	—	4–7 days	11–14 days	G. 13 ,, U. 8–20 ,, K. 30–45 ,,	57 ,, 153–216 ,, 281–351 ,,	2–3 ,,	3–4 ,,	2–3 ,,	8–10 days	U. 5·3 ,, K. 4·9 ,,	49 ,, 90 ,,
Guinea-pig ...	8 ,,	55 ,,	15 ,,	3–5 + mths.	—	—	12–45 ,,	3–8 mths.	G. 26 ,, U. 16 ,, K. 20 ,,	100 ,, 81 ,, 100 ,,	Always negative	1½ ,,	2–3 ,,	3–5 mths.	U. six experiments with no positive result K. only 2 positive results out of 21 experiments	—
Rabbit	8 ,,	28 ,,	12 ,,	3–5 + mths.	—	—	5–15 ,,	54–128 days	—	—	Always negative	1–4 + ,,	8–14 ,,	4–5 mths.	U. 9 ,, K. 9 ,,	28·6 days 49 ,,
Dog	4 ,,	12 ,,	14 ,,	More than 1½ months	22 ,,	Some months	5–10 ,,	33–43 ,,	—	—	17 days	66 days	2–3 ,,	1½–4 mths.	—	—
Monkey—																
Macacus rhesus	4 ,,	11 ,,	4 ,,	1–3 mths.	9–22 days	2–8 mths.	—	—	—	—	8–9 ,,	16 days to 2 months	—	—	—	—
Cercopithecus callitrichus	4 ,,	9 ,,	13 ,,	3–5 ,,	9–40 ,,	2½–12 ,,	—	—	—	—	7 ,,	19–88 + days	—	—	U. 10 ,, K. 12 ,,	82 ,, 105 ,,
Cercopithecus ruber	4 ,,	14 ,,	—	—	—	—	—	—	—	—	7 ,,	24 days	—	—	—	—
Goat	7 ,,	48 ,,	Parasites only found once on 19th day in Goat 1; never found in Goat 2	3–5 + mths.	Parasites found on 13th day after 5th inoculation in one goat. Other 2 goats negative	One animal died on 22nd day. Other two recovered	—	All died: duration not stated	—	—	—	—	—	—	—	—
Donkey	6 ,,	41 ,,	—	—	3 were inoculated, but did not become infected	—	Parasites found on rare occasions	—	—	—	—	—	—	—	—	—
Horse	7 ,,	38 ,,	—	—	—	—	30 days	Recovered	—	—	—	—	—	—	—	—

* G. signifies *T. gambiense*, from Gambia; U. Trypanosome of Sleeping Sickness in Uganda; K. Trypanosome of Sleeping Sickness in Congo.

My observations as to the morphology of the parasite confirm those of Stephens and Fantham regarding the existence of posterior nuclear forms in infected rats, guinea-pigs and rabbits. I have also found them in monkeys (*Macacus rhesus*, *Cercopithecus callitrichus*), dogs, mice, horse and donkey.

I have not, however, succeeded in finding these forms in the blood of the patient himself, in spite of very careful daily examinations during a period of over three months.

Why the posterior nuclear forms should be apparently absent from the patient's blood is not very obvious. Trypanosomes were always scanty and possibly these forms may have been missed, although nearly two thousand parasites were examined without meeting a single example of the form in question. However, the posterior nuclear variety is not usually seen in infected animals until the blood is fairly heavily infected, when they may form as many as five or six per cent. of the total parasites present.

Sections of the nervous system of the patient exhibit well-marked perivascular infiltration and leptomeningitis. The spleen was greatly increased in size and deeply pigmented (malarial?). The cervical, axillary, mesenteric and inguinal lymphatic glands were considerably enlarged, and presented the usual histological appearances.

ON THREE NEW SPECIES OF THE GENUS GLOSSINA, TOGETHER WITH A DESCRIPTION OF THE HITHERTO UNKNOWN MALE OF *GLOSSINA GROSSA*, BIGOT

BY

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(*Received for publication* 15 *December*, 1910)

The discovery of the three species of *Glossina* herein described was brought about by an examination of the morphological characters of the genital armature of the males. In the first instance some doubt had been entertained regarding the correct specific identity of certain dark forms of tsetse flies which had been placed in the collections of this School as doubtful examples of *Glossina morsitans*, West. The examples in question were captured by Major Dansey-Browning, in the Bahr-el-Ghazal Province of the Sudan, during the winters of the years 1905 and 1906. These captures were recorded* by him in the year 1908, with a note as to the peculiar dark colour which they presented in life. More recently a long series of tsetse flies, which were collected by Dr. F. W. McCay, on the Katangum River, between Katangum and Geidam, Northern Nigeria, were presented to this School. These examples were found to be specifically identical with those taken in the Sudan. On comparing the genital armature of these dark forms with those of typical *G. morsitans* from Rhodesia, it was found that they were structurally distinct; it became necessary, therefore, to raise the dark forms to specific rank. On the examination of some *western* examples of the so-called *Glossina morsitans* one found also that these presented the same morphological characters as those of the dark forms both from the Sudan and Katangum. My interpretation of these specific differences is that all the tsetse flies occurring in the western regions of Africa which have hitherto been

* Jour. Roy. Army Med. Cor., Vol. X, p. 427.

considered as *Glossina morsitans* will prove, on further examination, to be referable to the species herein described as *Glossina submorsitans*, n. sp.; what the exact distribution of this insect is remains to be seen, as at the present moment I have no further material available for examination.

The examination of the morphological characters of the armature of the males of all the other known species of the genus *Glossina* has revealed the fact that there are four distinct species of that group of tsetse flies, of which *G. fusca*, Walk., may be taken as a type. These are *G. longipennis*, Corti, and *G. brevipalpis*, Newstead, from the eastern side of the continent; while *G. fusca*, and *G. grossa*, Bigot, are, as far as I can gather at present, the western representatives of this group.

With regard to the large tsetse fly herein referred to as Bigot's *Glossina grossa*, I had at first thought this to be a new and hitherto undescribed species; but, on further consideration, I came to the conclusion that, as it agreed so well with the author's description,* it must be referable to this insect; all the more so on account of the statement made by Austen,† that 'the apex of the third joint of the antennae is very prominent.' Austen, who had evidently examined Bigot's type, did not, however, consider this marked character of specific importance, and sunk *Glossina grossa* as a synonym of *G. fusca*, Walk. Should my examples eventually prove to be specifically distinct from *G. grossa* I would suggest the specific name *nigrofusca*.

It may be interesting to add also, in this connection, that there are no structural differences between *Glossina palpalis* and the var. *wellmanni*; so that for strictly medical purposes I would suggest that the var. *wellmanni* should be sunk, all the more so seeing that *Glossina palpalis* is given to great variation in size, and that there are colour varieties or forms which are intermediate between typical *palpalis* and its var. *wellmanni*.

I have, so far, been able to examine but one specimen of *Glossina pallidipes*, Aust. This proves to be specifically identical with *Glossina longipalpis*, Wied. The most, therefore, that one

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* Ann. Soc. Ent. Fr., Vol. LX, p. 377 (1901). Female only.

† Monograph of Tsetse Flies, p. 97.

can do in the light of this discovery is, I feel, to make *G. pallidipes* a colour variety of *G. longipalpis*.

A more extensive paper dealing with the morphological characters of the genital armature of all the known tsetse flies is in the course of preparation, and will be published early in the new year, together with a series of illustrations which will aid the student in the exact determination of this genus of pathogenic insects.

Glossina submorsitans, n. sp.

Nearly related to *Glossina morsitans*, Westw., in having the tips of all the tarsi dark. There are two well-marked varieties: a *dark form* and a *light form*. The former may be readily distinguished by its general dusky or dull vinous-grey colour and generally smaller size; the pale form, however, very closely resembles *G. morsitans* in the general colour of the abdomen; but the bilateral abdominal bands are:—

(*a*) much more clearly and sharply defined.

(*b*) equally and more narrowly interrupted in the median line, between the *third*, fourth, and fifth segments.

(*c*) slightly rounded medially and suddenly tapering towards the lateral margins.

In *G. morsitans* the bands are:—

(*a*) not so sharply defined medially.

(*b*) gradually interrupted in the middle line, the space between the *third* being much greater than the space between the fifth, and the dark colour gradually shades off into the pale colour forming the median line.

(*c*) *broadly* rounded medially, and a very gradually tapering towards the lateral margins.

The morphological character of the genital armature of the males of these species, respectively, are easily separable by the form of the median processes.

DARK FORM.—*Thorax* grey, with the usual dark brownish longitudinal markings, generally relatively small, sometimes reduced to elongated spots. *Abdomen* dull olivaceous grey, with

a faint vinous tinge, most pronounced on the second and third segments; second segment with a large bilateral infuscated blotch, often enclosing a small but distinct sub-central blackish spot, but occasionally also the spots are separated, and in some examples the small dark one only remains; the third to the sixth segment, inclusive, with sharply defined and conspicuous blackish bands, narrowly interrupted in the median line. *Legs* with the tips of the last two segments of the front and middle tarsi black; the last two segments of the hind tarsi uniformly black.

Length.—7 to 8 mm.; length of wing, 7 to 8 mm.

Type, male and female (Coll. British Museum), Katangum River, between Katangum and Geidam, Northern Nigeria. Dr. F. W. McKay.

PALE FORM.—*Thoracic* markings similar to those in the dark form; but the pleurae are generally paler. *Abdomen* greyish buff, with a pale vinous tinge; bilateral blackish bands, very sharply and clearly defined and narrowly interrupted in the median line; those on the *third, fourth, and fifth segments* being almost equally separated by the pale median stripe; the bands are also only very slightly rounded medially, and rather suddenly so towards the lateral margins. *Legs* as in the dark forms.

Two co-type males and a female (Coll. Brit. Museum) were captured by Dr. A. Kinghorn at Sabiya-Boge, Northern Ashanti, 24th April, 1910. Eight additional specimens were also captured by Dr. Kinghorn in various localities in Ashanti, all of which have pale abdomens as in the types.

Glossina brevipalpis, n. sp.

This tsetse fly may be easily recognised from the two western representatives of the fusca group (*G. fusca* and *G. grossa*) by its shorter and stouter palpi, its generally paler colour, and somewhat indefinite thoracic markings; in the morphological characters of the male armature; also by the great length of the median process; the presence of a long bilateral row or band, of squamose spines; and in the form of the harpes.

Length.—10-12 mm.; length of wing, 11-11·50 mm.

MALE.—*Head* with the front uniformly pale ochreous brown, frontal margins brighter. *Antennae* pale ochreous, with the anterior

half infuscated; arista infuscated at the base. *Eyes* slightly converging. *Palpi relatively short and stout. Thorax* dark brown, with the markings dusky grey-brown, these vary in intensity, but are usually more or less indefinite.

Abdomen dark brown; second segment scarcely paler than the others; margins and posterior angles sometimes narrowly paler.

Legs pale ochreous brown; last two segments of middle and hind tarsi dark, blackish; last two segments of front tarsi either with or without brownish tips; front femora infuscated dorsally, and slightly so laterally on the inside; hind tibiae with a more or less distinct infuscated band; venter, in examples which have not partaken of a meal of blood, pale ochreous brown, margins of the third to the sixth infuscated; stigmata dusky ochreous.

FEMALE.—Colour pattern closely resembling that of the male, but with the hind and lateral margins generally more strongly pronounced.

Type, female, Songwe River, N. Nyasa, 7th January, 1909 (Dr. J. B. Davey). Co-type, male, near Kporo, N. Nyasa, 5th August, 1909 (Dr. J. B. Davey).

Glossina grossa, Bigot.

Ann. Soc. Ent. Fr., Vol. LX, p. 377 (1891). Female only.

This species is distinguishable from *Glossina fusca* by its generally darker colour; the darker hind tarsi in the male; the narrow lines on the thorax and the strongly pubescent character of the antennae. Morphologically also in the male armature by the form of the clasperettes, the large group of squamiform spines over the harpes, and the broad dark sclerite arising from its anterior portion near the base of the mantle.

Length.—11-12 mm.; length of wing, 11-11·50 mm.

Thorax very dark brown to blackish brown, with *very narrow pale longitudinal stripes, scarcely broader than the pale transverse suture. Abdomen* blackish brown, with the second (largest) segment markedly paler. *Legs* dusky; hind tibiae either all dark or with the last two black, the rest also rather dark but gradually paling proximally; hind and middle femora with a broad infuscated band.

MALE.—*Head* dusky brown to dusky buff. Posterior surface dusky grey, sometimes darker towards the upper margins; facial pit grey to ochreous grey; frontal margins shimmering creamy white or yellow white. *Antennae* with the terminal segment *strongly recurved, and clothed with long pubescent hairs*, the longest hairs on the dorsal edge being equal to about three-fourths the width of the segment. *Palpi* long, relatively thin and dark brown, tips slightly darker. Bulb of proboscis pale, with the proximal portion faintly infuscated.

Thorax.—Very dark brown to blackish brown, with very narrow, pale, longitudinal stripes, the sub-median ones being generally sharply and clearly defined, and scarcely broader than the pale transverse suture; pleurae dark brown.

Abdomen.—Blackish brown, with the second segment ochreous brown, there is no median stripe to the remaining segments, but a trace of ochreous brown is faintly visible on the third, fourth and fifth; lateral margins of all the segments narrowly paler than the rest; venter dusky ochreous; sides of the third, fourth and fifth segments, and also the hypopygium, infuscated; stigmata white.

Legs.—Yellowish brown or buff; femora broadly and distinctly infuscated, especially the middle and hind pair; middle and hind tibiae also with a very broad infuscated blotch; anterior tarsi with the tip of the fourth segment black, last segment dark at tip but not black; middle tarsi similar; hind tarsi all dark but the distal half of the third and the two succeeding ones all black; hairs on ventral surface bright golden brown.

The female differs from the male in having the legs decidedly paler; the hind tarsi with the last two segments black, the second and third with the tips only black. The thoracic and abdominal markings, and also the form and strongly pubescent character of the long terminal segment of the antennae are specifically the same as in the male.

Described from four examples, three males and one female. One male was taken at Kasongo, Congo Free State, 6th February, 1904 (Drs. Dutton and Todd), one at Sunyani, Ashanti, 5th March, 1910, and the third at Atroni, W. Ashanti, 16th August, 1910 (Dr. A. Kinghorn). The only female I have yet seen was taken at Odumase, W. Ashanti, 27th April, 1910, by Dr. A. Kinghorn.

Glossina fuscipes, n. sp.

This tsetse fly may be readily distinguished from *Gl. palpalis*, Rob.-Desv., by its much smaller size, by the uniformly infuscated or dusky legs, and the dusky grey thorax. In size it resembles *G. tachinoides*, West, but it is a relatively stouter built insect; and altogether it is most like a dwarfed specimen of *Gl. palpalis* with infuscated legs and a dusky thorax. The genital armature resembles those of *Gl. palpalis* and *Gl. tachinoides* in its general form; but the shape of the superior claspers and the inferior claspers are markedly distinct from those of either of these insects.

Length.—7½ mm.; length of wing, 8 mm.

MALE.—*Head:* Frontal stripe yellowish brown, margins dusky white; ocellar triangle buff, enclosing a very dark brown spot. *Antennae* grey; arista dark brown ventrally. *Palpi* smoky brown, with a paler narrow median line. *Proboscis* with the bulb dark castaneous.

Thorax with a median dark grey rectangular area extending from the front to the scutellum, the sides of which are perfectly straight and parallel, on either side of this grey area are two greyish-black, and somewhat triangular blotches, evidently remnants of those found in other species of Glossina; scutellum dark grey, with a very faint pale grey median line, margins buff; sides of the lateral thoracic depressions dark brown or almost black; pleurae dark grey.

Abdomen resembling that of *Gl. palpalis*, but the bilateral banding faintly pronounced; lateral margins with pale angular areas.

Legs strongly and almost uniformly infuscated (dark grey); femora with the basal and ventral portions ochreous buff; hind tarsi all dark brown or almost black.

Wings.—Anterior transverse vein strongly and suddenly incrassate at its junction with the third longitudinal vein.

HABITAT.—The only example which we possess of this interesting tsetse fly was taken by Dr. Shircore,* at Nimule, Nile Province, Uganda. Fortunately it proved to be a male, otherwise it may have passed as a small dusky form of *Glossina palpalis*.

* The data here given are taken from Dr. Shircore's letter; the actual locality is not stated by him. *R. N.*

DESCRIPTIONS OF A NEW GENUS AND THREE NEW SPECIES OF ANOPHELINE MOSQUITOS

BY

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AND

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(*Received for publication* 15 *December*, 1910)

DACTYLOMYIA, nov. gen. (Newstead and Carter)

(Figures 1-3)

Head.—Clothed with upright forked scales; palpi rather densely scaled.

Thorax (fig. 2).—With a *distinct and very pronounced cylindrical-shaped tubercle* or finger-like process projecting obliquely from the prothoracic region, mid-way between the dorsum and venter, and arising from the anterior margin. Thorax and abdomen clothed with hairs.

Wings.—With dense lanceolate scales. Judging by the character and distribution of the scales this genus comes near *Anopheles*; but owing to the remarkable structures mentioned above it is easily distinguishable from any other known genus of the *Anophelinae*.

Dactylomyia ceylonica, nov. sp. (Newstead and Carter)

Under pocket lens × 16.

Head.—Black in the centre, white in front; palpi brown, with three white bands and a few spots at the base, the apical band being the narrowest; proboscis with the apical half yellow, except for a minute black band near the apex.

Thorax and abdomen dark, covered with golden hairs.

Legs.—Brown, with the femora, tibiae and metatarsi spotted, the apex of the tibiae with a white spot; tarsi narrowly banded.

Wings.—With two small basal and four large costal spots, and many spots on the wing field; fringe with a large pale spot extending from the middle of the first fork cell to a short distance below the apex of the third long vein; there are other pale areas at the apices of the veins.

Microscopical characters.

Head.—Black, with a tuft of long white scales and several pale bristles projecting between the eyes; the rest clothed apparently with white, upright forked, scales in front and dark ones behind, but owing to its rather denuded condition we cannot be certain as to the exact nature and distribution of the head scales.

Palpi (fig. 1).—With the basal half clothed with brown scales, with the exception of three small spots formed by white ones; the apical portion with two broad bands of pure white scales, a narrow band of yellowish scales and short golden hairs at the apex.

Proboscis (fig. 1).—Distinctly curved at the base and clothed with deep brown scales on the posterior half, dull yellow ones anteriorly, and immediately before the labella are a few blackish scales, hardly sufficient in number to form a band.

Antennae.—Pale brown, the basal segment bare (? denuded), the first segment with many white scales.

Thorax.—Very dark brown, almost black, with a greyish tinge at the sides; hairs pale ochreous; thoracic tubercle (fig. 2) distinctly cylindrical in shape and gradually rounded in front; it is sufficiently prominent to be seen distinctly with a pocket lens; dark brown, shining on the denuded portion, sparsely clothed at the apex with fine pubescent hairs; sides apparently with minute greyish scales; scutellum dark brown with many long dark border bristles and also several shorter golden ones; halteres pale, the knobs clothed with small white scales.

Abdomen.—Dark brown covered with golden hairs.

Legs.—Deep brown; the femora, tibiae and metatarsi with white spots; tarsi of the first pair with narrow yellowish bands involving both sides of the articulations, last tarsus with dull ochreous scales; hind legs with apical yellow bands, the last tarsal segment, and metatarsus with a distinct apical band; mid legs wanting.

Wings (fig. 3).—Clothed with yellow and black lanceolate scales; costa with two small basal and four large spots, the two median ones being the largest; the first large spot extends evenly on the sub-costa and first longitudinal vein, the second extends evenly on to the sub-costa, but it is represented by two smaller spots on the first longitudinal; the third and fourth spots extend evenly on to the first longitudinal vein, the fourth also being continued to the upper branch of the first posterior cell. The second long vein has a spot just before the cross vein and a rather longer one immediately after; the branches of the cell, besides the spot on the upper branch mentioned above, have several scattered black scales. Third vein with six small sub-equal patches. Stem of fourth long vein completely dark scaled, base of the fork yellowish, upper branch with a black patch immediately after the fork and a few slightly darker scales towards the apex, lower branch with several dark scales, those nearest the base forming a small patch. Fifth vein with a patch near the base and six small, almost equidistant, spots on the remaining portion of the stem and lower branch of the fork, upper branch with four spots, the first one being just after the posterior vein. Sixth vein also with six small equidistant spots. Fringe with pale areas at the apices of the veins, that at the apex of the wing being the largest.

Length.—3·5 mm.

HABITAT.—Trincomalee, Ceylon (E. E. Green).

We are greatly indebted to our colleague Mr. E. E. Green, the Government Entomologist of Ceylon, for this extremely interesting and unique mosquito, and congratulate him on the discovery of such an interesting form. This specimen was presented to the Liverpool School of Tropical Medicine, November, 1907, together with a number of other Anophelines, and until quite recently its true generic position had been overlooked.

Pyretophorus cardamatisi, nov. sp. (Newstead and Carter)

(Figures 4 and 5)

Under pocket lens × 16.

Head.—Black behind, white in front with a projecting tuft of long white scales; palpi long and thin, black, with three white

bands, the apical one being the broadest; proboscis *very long and straight*, black with a pale apex.

Thorax.—Grey-brown clothed with whitish scales, with a dark median line and dark lateral ridges.

Abdomen.—Brown, with pale hairs.

Legs.—Brown, with pale articulations.

Wings.—Rather thinly scaled, with two small basal and four large costal spots; fringe with five pale areas.

Microscopical characters.

FEMALE.—*Head* black, with numerous white upright forked scales forming a more or less triangular group in front, the remaining portion covered with black forked scales, with the exception of a few on the posterior lateral margin, which are of a golden yellow colour; projecting between the eyes is a tuft of elongated white scales and several greyish bristles.

Palpi (fig. 4) and *proboscis* very long and thin, the former clothed with dark brown scales, with white bands at the articulations, and with a distinctly broader band at the apex, the latter covered with dark scales, except those on the labella, which are of a dull yellow colour.

Thorax.—Integument of the median dorsal area grey-brown, the sides dark brown, forming two more or less distinct stripes, scales on the pale area greyish white; pleurae paler than the lateral margins of the thorax, almost bare, but with a few scattered grey scales present.

Abdomen.—Pale grey-brown, with dark brown apical margins to the segments, and clothed with greyish hairs.

Legs.—Brown, all the articulations pale.

Wings (fig. 5).—With lanceolate scales on the veins. Costa with two small basal and four large spots, the latter approximately equidistant and sub-equal in length, all extending on to the first longitudinal vein, spaces between the spots clothed with yellowish scales. The scales on the first two-thirds of the stem of the second longitudinal vein chiefly black, the rest of the stem and base of the fork white; tip of upper branch yellow the rest black, lower branch black with the exception of a small area below the costal spot. Third vein almost entirely clothed with yellowish white

scales, except at the base and apex, which are covered with black scales. Basal two-thirds of the fourth vein yellowish, a black spot reaching nearly to the cross veins, then another black patch commencing just after the cross veins and terminating at the fork; base of the fork yellowish, upper branch with two nearly equal black patches and a median pale area; lower branch similar. Fifth long vein with a small black spot at the base, rest of the stem light, base of fork pale, upper branch with a few black scales before the cross vein, space near the latter with pale scales, followed by a patch of black scales then white ones, and distally with a few black ones; basal one-third of the lower branch pale, the rest black. Scales on the sixth vein all black with the exception of two small sub-median patches of white ones.

Length.—4 mm.

MALE.—*Palpi* of the typical Anopheline form with three pale bands; the first at the apex of the narrow portion; the second, which is much deeper ventrally, at the apex of the basal segment of the club; and the third at the extreme tip. The remaining characters as in the female.

Length.—3·5-4 mm.

HABITAT.—Athens (Dr. J. P. Cardamatis).

The specimens of this species were presented to this School by Dr. J. P. Cardamatis, November 18th, 1907, and we believe that they were captured by him in the summer of that year.

The distinctive characters of this Anopheline are the markedly long, thin, and straight proboscis, the banding of the palpi and the wing markings.

Cellia cincta, nov. sp. (Newstead and Carter).

(Figures 6 and 7)

Under pocket lens × 16.

Head.—White, dark posteriorly; proboscis black, apex yellow; basal portion of palpi dark with dense outstanding scales.

Thorax.—Greyish, with lateral ridges.

Abdomen.—Blackish grey, covered with golden hairs and with outstanding tufts of scales; apical segment with yellowish scales.

Legs.—Femora and tibiae with numerous yellow spots; metatarsi and tarsi with small alternating black and yellow rings; last tarsal joint in all the legs yellow.

Wings.—With five costal spots, the two median ones being much the longest; fringe with six pale areas.

Microscopical characters.

Head.—With a large and more or less triangular area clothed with white forked scales, these scales extend from the vertex to the extreme front, sides clothed with large dense black upright forked scales. Two or more long white bristles project over the clypeus. *Antennae*, basal segment greyish brown; a few of the succeeding segments dark brown, the rest greyish; short hairs silvery grey. *Palpi* (fig. 6*b*) clothed with rough outstanding dusky black scales; articulation of the first and second segments white scaled, in more perfect examples these will in all probability form a distinct ring; the basal portion of the second segment is also clothed with dusky black scales; the remaining portions of the palpi are unfortunately wanting.

Proboscis dusky black, with the labella yellowish white.

Thorax.—Prothoracic lobes clothed with long outstanding blackish scales, these are distinctly spathuliform in shape, with suddenly truncated apices. Thorax greyish brown, with white scales; on the front of the mesothorax these are very narrowly spindle-shaped, and when viewed obliquely appear as narrow curved ones, those on the lower portion of the thorax behind the wings are distinctly spindle-shaped and more dusky-white than those in front; sides of thorax in front greyish. Posteriorly the pleurae are brownish, and with a few scattered white scales. Halteres with a greyish stem, apex darker with a few brown scales.

Abdomen.—Distinctly dark slaty grey; hairs pale; apical margins with dense dark brown outstanding scales, intermixed with these, dorsally, are a few greyish white ones; apical segment clothed chiefly with yellowish white scales; venter blackish, with pale hairs.

Legs (fig. 6*a*).—Middle and hind femora and tibiae very conspicuously spotted with yellowish white scales, so distinctly so, as to appear almost ringed; knees yellowish white; metatarsi and

tarsi with distinct and equidistant pale yellowish rings; terminal segment uniformly yellow.

Wings (fig. 7).—Clothed with black and yellow lanceolate scales; costa with five spots, all extending on to the first long vein, the third spreading evenly on to sub-costa, but interrupted on the first long vein by a group of yellowish scales near the proximal portion. Second vein with black patches of scales immediately before and after the cross vein, upper branch of the fork except at the base and extreme apex black scaled, lower branch with a pale spot at the base, then a short black patch, and the remaining portion clothed with pale scales, some of which are tinged with black. Third vein entirely yellow scaled, with the exception of a few black scales at the extreme base and apex. Stem of the fourth longitudinal vein with a large spot just before the fork, a few black scales behind the posterior cross vein and at the base; upper branch with two spots, lower branch with one median dark patch. Fifth long vein with a few black scales at the base of the wing, the rest being yellow; upper branch with a small black patch at the base, a larger one at the apex and another in the middle; lower branch completely yellow scaled, except for a small black spot at the apex. Sixth vein with a yellow patch towards the base, the remaining portion black scaled. Fringe with pale areas at the apices of the veins.

Length.—3 mm.

HABITAT.—This interesting Anopheline was captured by Dr. A. S. Donaldson at Broomassie, W. Africa.

It is markedly distinct from any other hitherto described species of *Cellia*, and may be readily distinguished by the very conspicuous and regularly disposed rings on the mid and hind legs. It may be further distinguished also by the rough outstanding scales on the palpi. The only specimen which we possess is in a splendid state of preservation so far as it goes, but, unfortunately, both the anterior legs and the apical segments of the palpi are wanting.

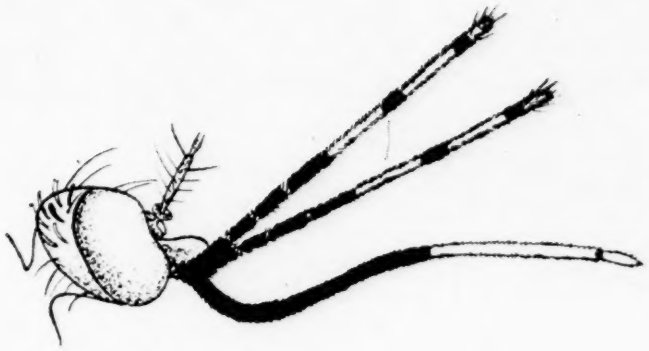

FIG. 1. Head of *Dactylomyia ceylonica*.
(Newstead and Carter.)

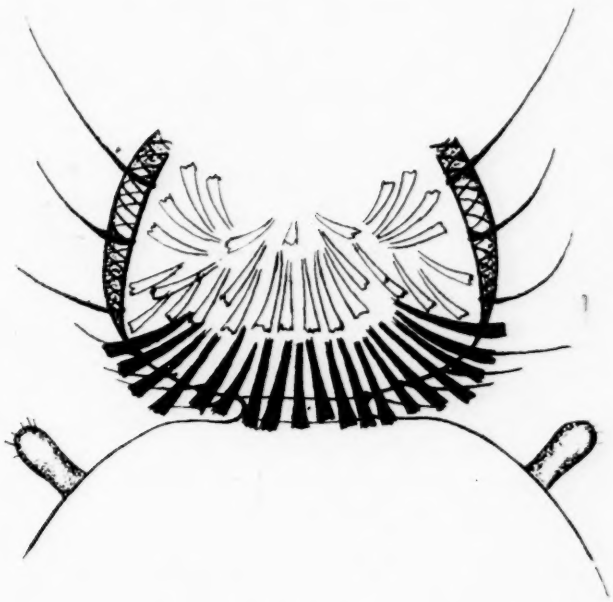

FIG. 2. Portion of thorax, showing tubercles, of *Dactylomyia*. (Newstead and Carter.)

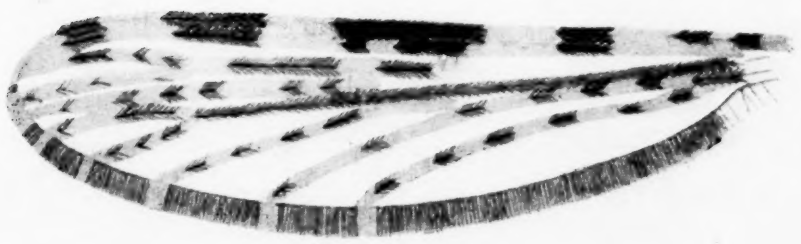

FIG. 3. Wing of *Dactylomyia ceylonica*.
(Newstead and Carter.)

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ERRATA.

Plate XXIV, figs. 6, 7.

For *Cellia annulipes* read *Cellia cincta*.

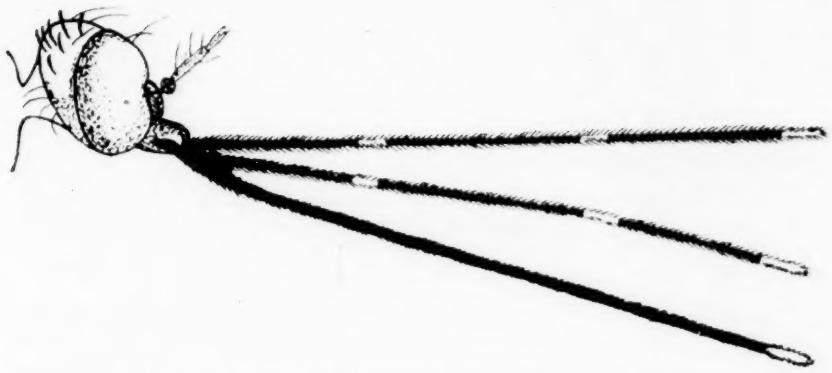

FIG. 4. Head of *Pyretophorus cardamatisi* ♀
(Newstead and Carter.)

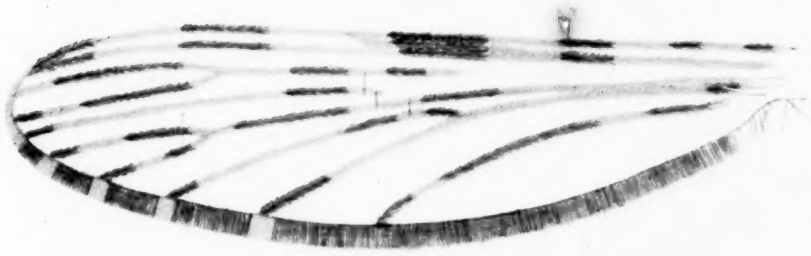

FIG. 5. Wing of *Pyretophorus cardamatisi*.
(Newstead and Carter.)

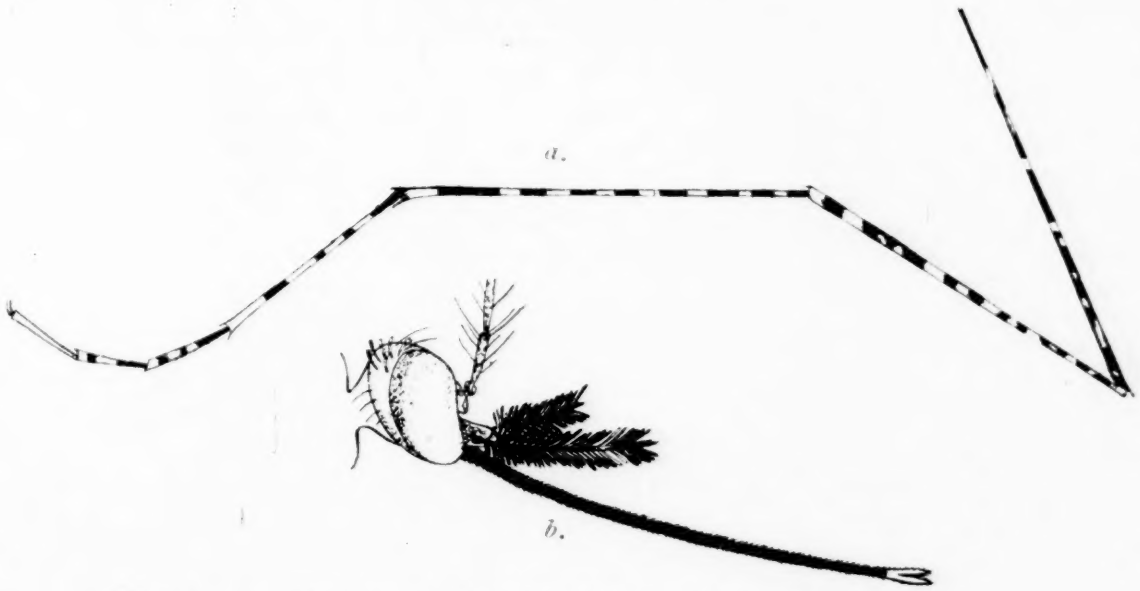

FIG. 6. *a*. Hind leg of *Cellia annulipes*. (Newstead and Carter.)
b. Head of *Cellia annulipes*; anterior portion of palpi wanting.

FIG. 7. Wing of *Cellia annulipes* ♀ (Newstead and Carter.)

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